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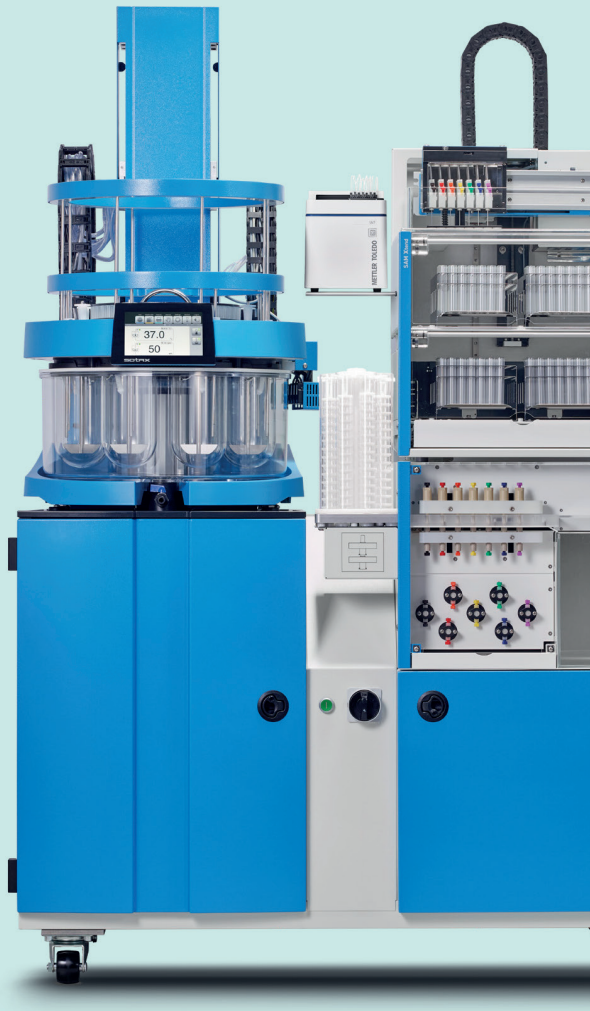

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The **February 2024** issue will include research articles on topics of biphasic dissolution, controlled release caffeine capsules, acetaminophen overdose model, and similarity of montelukast products. This issue will also include the Dissolution Performance Verification Standard (DPVS) webinar report, AAPS 2023 annual meeting highlights, and the Q and A feature.

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Publisher's Information

PURPOSE: *Dissolution Technologies* is a peer-reviewed quarterly publication reporting current information on dissolution testing. It provides an international forum for open exchange of information on various dissolution topics.

CORRESPONDENCE: Send all correspondence to: *Dissolution Technologies*, Inc., 9 Yorkridge Trail, Hockessin, DE 19707. Phone (302) 235-0621, e-mail: vagray@rcn.com. Website: www.dissolutiontech.com

STAFF: Founder, Cynthia Brown; Managing Director, Vivian Gray; Associate Editor, Valerie Clark; Research Editor, Vivian Gray; Research Editor, William Brown; Communications, Michael Larson; Circulation Manager, Sandra Larson; Layout, Michele Arnold; Publication, Printing, and Distribution Services, Archer Print Group, Sellersville, Pennsylvania.

ISSN 1521-298X

Dissolution Technologies is indexed in:
Science Citation Index Expanded®
Journal Citation Reports®/ Science Edition
International Pharmaceutical Abstracts
Chemical Abstracts (CAS)
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STIMULI TO THE REVISION PROCESS

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Testing the In Vitro Product Performance of Mucosal Drug Products: View of the USP Expert Panel

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ABSTRACT

Performance testing of mucosal drug products presents the user with a multitude of challenges. Not only are there many different dosage forms to be distinguished, but also a wide variety of administration routes. The target action effect (local or systemic) is another factor to be considered. Thus, it quickly becomes apparent that there will never be a universal performance test, but the question arises just as quickly whether the method to be used should rather depend on the dosage form or the place of application, or even whether decisions must be made on an individual basis. This *Stimuli* article is one of a series of *Stimuli* articles on product performance testing, which focuses on methodological approaches and challenges in the field of performance testing of mucosal drug products. The article should be viewed as a supplement to, but also a critical discussion of the methods listed in USP general chapter *Mucosal Drug Products—Performance Tests* <1004>. With consideration of major physiologic aspects at the site of administration and the types of dosage forms to be studied, limitations of the methods described here and the need for methodologic updates or innovations are identified. Furthermore, suggestions are made for future activities, all aimed at developing robust, discriminatory, and meaningful test methods for the wide variety of mucosal drug products.

INTRODUCTION

The development and application of appropriate in vitro performance tests is one of the cornerstones of quality assurance of pharmaceutical dosage forms. In recent decades, there has been a considerable increase in the number of corresponding test methods in international pharmacopoeias, including the United States Pharmacopoeia (USP). It is noteworthy that even though all of the test methods developed to date have basically had the same objective, namely, to ensure the quality, safety, and efficacy of the medicinal product in question, older methods are often relatively simple and, in some cases, do not really show a direct link to the administration site and drug target action site. Nevertheless, they serve the purpose for which they were designed, which is to ensure critical quality measures for the products they were designed for. However, the question arises as to whether the methods in question also accomplish this when applied to similar products intended for the same application.

For many years, we have witnessed a growing number of new pharmaceutical entities and the development of novel dosage forms and generic medicines for a wide range of indications. Along with the increase in novel dosage forms and generic product development there is also an increase in the knowledge about the physiological conditions that may have an influence on the in vivo performance of a pharmaceutical drug product at the site of application and/or the site of drug release. Therefore, it seems reasonable to reconsider existing in vitro performance tests regarding their capabilities and significance, to identify possible methodological gaps, and to think about modern methods that are meaningful,

differentiated, robust, and standardizable and, in the best case, not only provide information on quality but can also provide valuable information on the performance of the drug product (using in vitro or in vivo methods) under investigation.

This article is the sixth in a series of *Stimuli* articles from the USP Expert Panel on New Advancements in Product Performance Testing (EP-NAPPT) and was prepared by the Mucosal Drug Products subgroup. It aims to raise awareness of current practices and new developments in the evaluation of mucosal drug products. The basis for this article is *USP* general chapter *Mucosal Drug Products—Performance Tests* <1004>, which contains the current compendial product performance tests for drugs intended to be delivered to the body via the mucosal route.

The objective of this article is to:

- Evaluate current testing methods and perform a gap analysis that identifies the limitations and analytical challenges of current methods
- Indicate whether there is a need to update existing methods or to implement new performance tests for the various subtypes of mucosal drugs
- Propose methodological approaches for new product performance testing
- Facilitate public comments from users and regulators
- Gather comments from users and regulators and then draft new compendial chapters or update existing compendial chapters

MUCOSAL DRUG DELIVERY

Mucosal drug products deliver active pharmaceutical ingredients to the body via a vast variety of mucous membranes. These include the otic, ophthalmic, nasal, oropharyngeal, urethral, vaginal, rectal, and, in principle, also the pulmonary mucosa. However, USP clearly delineates the latter site of administration from all others and discusses dosage forms that refer to the pulmonary route of administration in *USP* test chapter *Inhalation and Nasal Drug Products—General Information and Product Quality Tests* <5>. The group of mucosal drug products discussed in *USP* chapters *Mucosal Drug Products—Product Quality Tests* <4> and <1004> is therefore limited to the remaining seven mucosal surfaces, with consideration given to products with both local and systemic effects. When considering the specified application sites for mucosal drug products, it quickly becomes apparent that the mucous membranes in question are located in very different locations of the body, which can differ significantly in their structure and function. It is basically a logical consequence that the different sites of application and therapeutic modalities result in quite different requirements for the mucosal drug products to be administered, which in turn directly indicates that the individual performance tests will probably have to be designed differently if meaningful results are to be obtained. These requirements also include whether the drug product is for a local or a systemic effect and whether the dosage form should demonstrate a rapid, delayed, or sustained drug release. As a rule, product performance tests are in vitro drug release studies. However, as noted in *USP* <1004>, consideration should also be given to whether alternative testing strategies (in light of the latest developments in the field) can provide the desired information.

GAP ANALYSIS

To assess the current state of science, to evaluate the possible need for novel in vitro methods, and also to evaluate the need for standardization of existing performance tests, the Mucosal Drug Products Subgroup of the EP-NAPPT performed a gap analysis for each individual subgroup of mucosal dosage forms. In addition to the general performance tests monographed in the *USP* chapters *Dissolution* <711>, *Drug Release* <724>, and *Semisolid Drug Products—Performance Tests* <1724>, individual product-specific USP performance tests, performance tests recommended by the Division of Bioequivalence of the US FDA's Office of Generic Drugs and listed in the FDA's Dissolution Methods Database, and methods listed in the scientific literature for the respective dosage forms were reviewed. In the following sections, the results of this gap analysis are discussed for each individual administration route.

Ophthalmic Route

Background

As stated in the introductory section of *USP* chapter *Ophthalmic Products—Quality Tests* <771> , the routes of administration of ophthalmic products fall into three general categories: topical, intraocular injections, and extraocular injections. Taking a more detailed look, a variety of individual routes of administration can be distinguished, including the topical, subconjunctival, subtenonal, subretinal, subchoroidal, intracorneal, intrascleral, suprachoroidal, intravitreal, intracameral, juxtascleral, and retrobulbar administration. Accordingly, ophthalmic products are administered to the eye in a

wide variety of dosage forms, including but not limited to solutions, suspensions, ointments, gels, emulsions, strips, injections, inserts, and implants. Currently, there are approximately 710 ophthalmic products including 470 generic versions and 320 discontinued products listed in the FDA's *Orange Book* (Approved Drug Products with Therapeutic Equivalence Evaluations).

The focus of this *Stimuli* article is on topically administered ophthalmic products. Whereas intra- and extraocular injections are administered through external boundary tissue, topical drug products are intended to be administered to an ocular surface component, such as the eyelid, conjunctiva, or cornea, and can produce local or systemic effects.

The anatomy and physiology of the eye are extremely complex. The eyeball, which weighs on average about 7.5 g and is about 24 mm long, consists of an outer layer with the cornea as the most anterior tissue layer of the eye, a middle and an inner layer as well as three internal sections, i.e., the anterior and posterior eye chamber and the vitreous body. In addition, various adnexa, especially upper and lower eyelid, lacrimal gland, and the lacrimal drainage system are important for ocular physiology and can significantly affect topical ocular drug therapy. Typical indications for topically applied ophthalmic drug products are the treatment of dryness and irritation of the eye, high intraocular pressure for glaucoma, and inflammation of the conjunctiva (conjunctivitis) and cornea (keratitis). Many of the drugs administered in this way are intended to act in the precorneal area of the eye or in the anterior part of the inner eye, but topical ophthalmic instillation is currently also being discussed as a strategy for delivering drugs to the back of the eye (*1*).

Administration of topical ophthalmic drug products to the cornea means application onto a membrane covered with a very thin film of tear fluid. Tear fluid is a buffered liquid containing a variety of components with a mean pH value of 7.2–7.4 and is approximately iso-osmolar with blood. The average volume of tear fluid available in the precorneal space is small, averaging 7 µL, of which approximately 1 µL is distributed over the cornea and 3 µL is located in each of the tear margins (*2*, *3*). Most of the tear fluid is produced in the lacrimal glands and drains into the nasal cavity via lacrimal ducts at the corner of the eye. Fluid hydrodynamics are influenced by blinking, among other factors. The average turnover rate of tear fluid is reported as about 15% × min^{-1} (*4*). The maximum amount of fluid that can be held in the cul-de-sac is 25–30 µL (*3*). Particularly for liquid formulations, the dose volume that can be administered is thus very low. Application of a drug product into the precorneal area typically induces tear flow. Therefore, not only the applicable dose but also the precorneal residence time of drugs after application is very limited, which severely limits not only the local availability but also the amount of drug that could penetrate through the cornea. Consequently, the ocular availability of topically applied drugs is usually low. With this information, it should be evident that the unique anatomy and physiology of the eye, and the numerous modes of drug delivery to the eye, pose a major challenge to the development of performance tests for topical ophthalmic drug products.

Performance Tests

Ophthalmic dosage forms include emulsions, gels, inserts, lenses, implants, ointments, solutions, and suspensions. Conventional dosage forms (e.g., solutions, suspensions, emulsions, and ointments) cover approximately 97% of the marketed topical ophthalmic products approved by the FDA. Ocular implants are typically administered by the intravitreal route and are thus discussed in the *Stimuli* article *In-Vitro Product Performance of Parenteral Drug Products: View of the USP Expert Panel* (*5*). The same applies to all liquid formulations for intra- or extraocular injection. Information on performance tests for ophthalmic products that have an extended-release (ER) mechanism (beyond 1 day), for which the dissolution or drug release rate is rate limiting for absorption and is expected to provide a controlled therapeutic response is provided in *USP* general chapter *Ophthalmic Products—Performance Tests* <1771>. The performance tests for all other ophthalmic drug products are listed in *USP* <1004>. *USP* <1724> is referenced for testing emulsions, gels, and ointments, i.e., official apparatuses such as the immersion cell apparatus and the vertical diffusion cell, which are commonly applied in performance testing of topical drug products, can be used. For emulsions, also *USP* Apparatus 2, the paddle apparatus can be used, and drug release of suspensions can be assessed with either the paddle apparatus or a miniaturized version thereof. The test conditions are not further specified. Although these test methods might be appropriate for quality testing of selected topically applied suspensions, emulsions, ointments, and inserts, it is questionable whether they would constitute meaningful performance tests. Furthermore, it should be noted that the currently described in vitro performance tests are considered inadequate for in situ forming gels and mucoadhesive formulations because these dosage forms interact with the mucosal membrane to exert their function or effect

in vivo, which can hardly be simulated with the standard set-ups described.

Another point to consider is that many drug products applied in the precorneal area of the eye are formulations that must show a rapid drug release because of the precorneal clearance. If differences in product quality that affect in vivo performance are to be detected in in vitro release testing (IVRT), the rate of drug release within a short release time period must be monitored as accurately as possible. Conventional sample-and-separate methods are often not suitable for this purpose. In standard set-ups such as the paddle apparatus with automated or manual sampling, one would simply not be able to take a sufficient number of samples within the time period of interest, and in diffusion-controlled test models, diffusion rather than release would be the rate-determining step for such rapidly releasing formulations. Accordingly, as part of method development and standardization, consideration must be given to the development of a fit-for-purpose and robust IVRT method that can reliably capture the released drug fraction even over very short time periods, to detect and distinguish variations in product quality and performance. For complex products (e.g., emulsions, suspensions, liposomes, drug-protein complexes), which are not limited to ophthalmic drug products only, an adaptive perfusion method, representing a pressure-driven separation method based on the principle of tangential flow filtration has recently been proposed for this purpose (*6*). It would certainly be valuable to evaluate this or similar methods for their universal applicability in in vitro performance tests for such dosage forms.

Table 1. Ophthalmic Drug Products Listed in USP <4> and Current USP Performance Tests According to USP <1004> and <1771>

Dosage Form, <4>	Performance Test, <1004> and <1771>
Emulsions	<711>, Apparatus 2, <1724> VDC
Gels	<1724>
Inserts and Lenses	<711>, <724>
Implants	<711>, <724>
Ointments	<1724>
Solutions	—
Strips	—
Suspensions	<711> Apparatus 2*

**Standard or miniaturized version.*
VDC, vertical diffusion cell.

Interestingly, despite the many ophthalmic drug products available on the market, USP does not currently list a single product-specific monograph that includes a requirement for an IVRT. The same situation is seen when reviewing the current FDA Dissolution Methods Database. Here, too, there are no specifications for an IVRT for an ophthalmic drug product.

Biorelevance of In Vitro Test Conditions

Current performance testing for topical ophthalmic products is not biorelevant due to the lack of consideration of available fluid volumes, composition, and dynamics (e.g., precorneal clearance) in the IVRT design. When it comes to studying topically applied dosage forms for drug delivery into the anterior part of the inner eye, it would be important to assess drug permeation through the cornea. Hence, this would have to be appropriately assessed in the IVRT. Currently, there is limited guidance on methods for evaluating corneal and conjunctival drug penetration in diffusion cell and permeation assays, e.g., criteria for tissue selection, tissue preparation, membrane loading with drug, receptor or dissolution media, and agitation or flow rate to be used in such experiments. In some areas, such as the development of artificial tears for in vitro studies, some progress has already been made in the past (*7, 8*). However, the focus of most of the reported studies was not on the development of an IVRT. Nevertheless, the method designs used in these studies as well as the experience gained, could be helpful for the discussion regarding the development of biorelevant in vitro performance tests for topically applied ophthalmic drug products. Although it is expected that there will be more efforts in the future regarding the development of biorelevant IVRTs for topically applied ophthalmic drug products, it should also be noted that for this sensitive application area in particular, there will invariably be differences between in vitro and in vivo conditions, so there is always the chance that performance tests may not be sensitive enough to detect differences in critical material properties and changes in critical manufacturing parameters that would affect in vivo performance.

In Vitro-In Vivo Correlation (IVIVC)

Just as there is a lack of biorelevant performance tests for topical ophthalmic drug products, there is also a lack of those that allow the development of IVIVCs for different ophthalmic dosage forms based on their routes of administration or the regions to which they are to be delivered. There are a few initial approaches in the literature that have been used, for example, to establish an IVIVC between the in vitro release of ocular inserts and their in vivo drug release in the conjunctival sac of rabbits (*9*). However, this was a formulation with release that probably depends little on the conditions prevailing at the site of application; the latter were not addressed in all relevant details the in vitro experiment and the amount of drug released was extrapolated solely from

the unreleased fraction of the administered dose (*9*). As the drug clearance and the distribution of the drug into different eye tissues were not taken into consideration, this developed IVIVC may not be able to predict in vivo performance of these ocular inserts (*10*). Overall, although a few attempts towards establishing IVIVCs have been reported, it can thus be concluded that there is a lack of suitable methods for IVIVC.

Otic Route

Background

The ear is divided into three parts: the external ear, middle ear, and inner ear. The external ear is composed of the pinna and external auditory meatus (ear canal). The ear canal is approximately 0.7 cm in diameter and 2.5-cm long (*11*) and provides passage from the outside to the tympanic membrane, which separates the external ear from the middle ear. The surface of the ear canal is lined with skin. The skin in the ear canal has short hairs and apocrine and sebaceous glands that produce ear wax. Earwax consists of fatty acids, fatty alcohols, squalene, and cholesterol and sometimes dead skin cells and hairs (*12*). The pH in a healthy ear canal is slightly acidic (pH 5–6) and increases with disease (*13*, *14*). The outer epidermal layer is continuous with the epidermis of the external canal. The diffusion of drugs administered to the ear canal into the air-filled middle ear cavity (and the inner ear) is controlled by the tympanic membrane (*15*). The tympanic membrane is a thin, cone-shaped membrane with a surface area of about 80 mm^2 and a thickness of 100–150 µm, depending on the location within the membrane. The tympanic membrane has a low permeability to most substances and its outer epidermal layer has similar properties to the stratum corneum (*15*). The shape of the external canal does not allow clear visualization of the tympanic membrane for monitoring drug application to the middle ear. Infection, trauma, or rapid pressure changes may cause perforation of the tympanic membrane. This creates a connection between the external auditory canal and the middle ear, and drugs from ototopical application can enter the middle ear. Diseases of the external ear requiring topical treatment are mainly associated with skin disorders. However, noninvasive trans-tympanic delivery of drugs to the middle ear has also gained interest, such as for instance for the administration of protective agents to treat drug-induced ototoxicity (*16*) or of antibiotics for otitis media treatment (*17, 18*). Topical antibiotics, corticosteroids, and anesthetics are commonly used for ototopical treatment and topical solutions (e.g., ear drops), suspensions, and ointments are typical dosage forms that are applied to the skin at the pinna, ear canal, and tympanic membrane.

The FDA *Orange Book* currently lists approximately 73 otic drug products including 54 generic versions and 40 discontinued products in the US market.

Performance Tests

Dosage form classifications of otic products can be found in *USP* general chapter *Pharmaceutical Dosage Forms* <1151>. Most of the marketed drug products are topical otic solutions (drops) and other dosage forms are suspensions and ointments. Because of the site of application and the nature of the dosage forms, the general test methods for otic drug products are similar to those for other topical products such as topical suspensions or ointments (see Table 2). For example, in vitro release set-ups for topical dermatological dosage forms such as the vertical diffusion cell or immersion cell apparatus can be used.

Table 2. Otic Drug Products Listed in USP <4> and Current USP Performance Tests According to USP <1004>

Dosage Form, <4>	Performance Test, <1004>
Ointments	<1724>
Solutions	—
Suspensions	<711>, Apparatus 2*

**Standard or miniaturized version.*

The FDA Dissolution Methods Database lists three otic suspension products (ciprofloxacin hydrochloride and hydrocortisone, ciprofloxacin and dexamethasone, and finafloxacin), but no in vitro release method is provided for these products. It is stated that methods will need to be developed to characterize in vitro release of these products. Beyond that, recommendations on performance tests for otic products are currently not available in the literature.

Methodological Standardization

As there are no official release testing methods for topically applied otic drug products so far, the question of how to standardize them does not arise. At the same time, however, this provides an opportunity to develop standardized methods from scratch that are preferably biorelevant, whereby a distinction must certainly be made between products that are generally applied in the ear canal and those that are applied directly to the tympanic membrane.

Biorelevance of In Vitro Test Conditions

The test procedures for otic drug products present similar issues as those for topical dermatological dosage forms. In both cases, the formulations are not in contact with

(a significant amount of) liquid after application, so that all methods in which the active ingredient release from the dosage form is investigated in direct contact with an aqueous medium are rather questionable because such conditions are very different from those at the application site. Thus, the development of biorelevant test methods can certainly be guided by methods for topical drug products for cutaneous application. However, situations specific to the ear such as the presence of earwax and the enclosing environment in the ear canal cannot be easily represented. In addition, when it comes to developing biorelevant in vitro performance tests for preparations for trans-tympanic drug delivery, one should keep in mind that the healthy middle ear is an air-filled space and that there is no liquid volume available for the active ingredient penetrated through the tympanic membrane in which it can disperse. Overall, it is important to discuss whether IVRT is the ultima ratio in the case of topically applied otic drug products when it comes to developing a meaningful performance test.

In Vitro-In Vivo Correlation

Currently, no biorelevant performance tests exist for otic drug products. As there has been no objective to predict the bioavailability of otic drug products on the basis of IVRTs, there also have been no approaches to establish IVIVC for such formulations.

Nasal Route

Background

The nose belongs to the upper airways and has, among other things, the task of warming, cleaning, and humidifying the air we breathe. Anatomically, it is divided into the external nose and the internal nose, and the two nostrils form the entrance to the inner nose. This comprises the nasal cavity, which is separated by the nasal septum into two, ideally symmetrical halves, the left and right nasal cavities. Inside them, the nose is lined with a well perfused mucous membrane with a surface of about 0.01 m^2. The main part of this mucosa, the respiratory mucosa, consists of three tissue layers: the top layer of which is the multilayer ciliated epithelium and contains glands that produce the nasal mucus (~40 mL × h^{-1}) protecting the nasal mucosa from drying out. Slightly different information on nasal mucosal pH can be found in the literature, which among other factors can certainly be attributed to the method used for determining the pH value (*19*–*21*). Overall, however, all studies indicate that the nasal mucus in healthy conditions is approximately neutral or very slightly acidic, whereby it was observed that the pH value increases with increasing distance from the nostrils (*20*). The nasal mucus is permanently moved towards the nasopharynx by the concertized back-and-forth movement of the fine cilia of the epithelium. The respiratory nasal mucosa typically represents the site of application for topically administered mucosal dosage forms, whereas the olfactory mucosa, located in the uppermost part of the nasal concha, is reached only by gases, vapors, and aerosol particles.

Most topically applied nasal medicines are used to achieve a local effect, e.g., in the treatment of colds, with vasoconstrictive agents for decongestion or immunologically active drugs predominating. However, intranasal administration also represents an interesting route of administration to deliver drugs into the bloodstream. The bypass of the hepatic first-pass effect, noninvasive application, good bioavailability, and rapid onset of action theoretically offer several advantages for selected drugs, which is why an increase in research activities in this area has been observed in the recent past. To date, 162 nasal drug products including 100 generic versions and 57 discontinued products reached the US market (FDA *Orange Book*).

Performance Tests

The currently official version of *USP* chapter <4> classifies nasal drug products into aerosols, gels (jellies), ointments, sprays, and solutions. The performance tests of these dosage forms are described in *USP* <1004>. Performance tests for nasal aerosols and nasal sprays are largely concerned with droplet or particle size distribution and aerodynamic size distribution. Accordingly, the procedures in the *USP* general chapter *Inhalation and Nasal Drug Products: Aerosols, Sprays, and Powders—Performance Quality Tests* <601> can be applied to products administered by mucosal routes. Performance tests of these formulations are thus part of the *Stimuli* article, *Testing the In Vitro Product Performance of Inhalation and Nasal Drug Products: Views of the USP Expert Panel* (*22*), and will thus not be discussed in the present *Stimuli* article. Aerosols, sprays, and solutions represent the vast majority of nasal drug products currently on the market, whereas only two gels and one ointment for nasal use are listed in the *Orange Book*. The general test methods for the latter two formulation types are similar to those for other topical gels or ointments (see Table 3). For example, in vitro release set-ups for topical dermatological dosage forms such as the vertical diffusion cell or immersion cell apparatus can be used. Beyond this general information, USP does currently not contain any product-specific monographs with information on in vitro performance testing of nasal gels and ointments. The FDA's Dissolution Methods Database also does not list

any product-specific IVRTs for nasal drug products, and no official recommendations on performance testing for nasal drugs can be found in the literature either.

Table 3. Nasal Drug Products Listed in USP <4> and Current USP Performance Tests According to USP <1004>

Dosage Form, <4>	Performance Test, <1004>
Aerosols	<601>
Gels (jelly)	<1724>
Ointments	<1724>
Sprays	<601>
Solutions	<601>

Methodological Standardization

As there are no official test methods for assessing the active ingredient release of nasal gels and ointments to date, the question of their standardization cannot arise. However, it should be clear that in future developments, care should be taken to establish methods that are as biorelevant and standardized as possible.

Biorelevance of In Vitro Test Conditions

For nasal drug products, there are similar issues as already discussed for ophthalmic and otic drug products. Here, too, the formulations will not be immersed in liquid after application, so that all methods in which the release of active ingredient from the dosage form is investigated in direct contact with large volumes of aqueous media are out of the question. There are already some published approaches in which electrolyte solutions of different compositions with slightly acidic pH have been used to simulate the composition of nasal mucus in an in vitro release experiment. In the corresponding experimental designs, a dialysis-based test design was used in which very small media volumes were employed (*23*–*25*). This could be a possible step toward more biorelevant test methods. A starting point for developing biorelevant test methods for nasal drug products could certainly also be based on methods for topical drugs for cutaneous application, but in any case, it should be borne in mind that, to circumvent mucociliary clearance by increasing retention time at the nasal mucosal surface, research is also increasingly being directed towards mucoadhesive preparations for nasal application, and a distinction must certainly be made between "ordinary" and mucoadhesive preparations when developing a biorelevant performance test.

In Vitro-In Vivo Correlation

Currently, there is no biorelevant performance test for semisolid nasal drugs. Therefore, it is understandable that no approaches to establish an IVIVC for such formulations have been published so far.

Oropharyngeal Route

Background

Oropharyngeal drug products are a class of mucosal drug products that deliver drugs to the mucosal surfaces within the oral cavity and are intended for either local or systemic action. Among the 100–200 cm^2 surface area of the intra-oral mucosa, the buccal (cheeks, gingivae, and inner lips) and sublingual (floor of the mouth and ventral side of the tongue) regions are most permeable for drug uptake or absorption through the nonkeratinized, 0.1- to 0.6-mm-thick stratified squamous epithelial cell barriers, and thereby, have most often been used for systemic drug delivery (*26*, *27*). Although the surface area of these main application sites (~80 cm^2) is smaller, when compared to the gastrointestinal (GI) tract (~200 m^2) or skin (~2 m^2) region, the buccal and sublingual mucosa represent highly vascularized regions that have direct access to the systemic blood circulation via the jugular vein. As a result, bioavailability of buccally or sublingually administered drugs can be high because hepatic first-pass metabolism is bypassed, whereas absorption can be rapid for certain lipophilic, low-molecular-weight drugs, as with injection (*26*, *27*). However, salivary fluid (pH 6.5–7.7) (*28*) is constantly secreted at 1–2 L/day or 0.7–1.4 mL/min, which eliminates dissolved or dispersed drugs to the GI tract by swallowing, and thereby reduces absorption via the oral mucosa. Swallowed drugs can then be absorbed from the GI tract, which may complicate interpretations of the systemic pharmacokinetic (PK) profiles (*26*, *27*). To date, about 210 oropharyngeal drug products including generic products are listed in the *Orange Book*.

Performance Tests

According to *USP* <4>, oropharyngeal drug products are classified into buccal patches, films, gels, gums, lozenges, ointments, solutions (rinses), sprays, and tablets. Note, however, that a revision for <4> has just been proposed in *PF* 48(5) for this classification. The performance tests of these dosage forms are described in *USP* <1004> as per the dosage form types across mucosal drug products rather than the routes of administration. As shown in Table 4, the performance tests for oropharyngeal drug products concern assessments of drug dissolution or release from dosage forms determined by the methodologies in existing general chapters for other (e.g., oral or transdermal) drug products with adaptations, and otherwise, are left unstipulated. *USP* chapters <711> and <1724> are referenced for films, gels, lozenges, ointments, and tablets, and drug release testing devices

described in *European Pharmacopoeia* (*EP*) chapter 2.9.25, Dissolution Test for Medicated Chewing Gums are referred to for gums. However, because the test methods in these chapters were not developed and validated for oropharyngeal, but other (e.g., oral or transdermal) drug products, relevant methodological adaptations are needed, as guided by the *USP* general chapter *The Dissolution Procedure: Development and Validation* <1092>. For drug release testing of sublingual tablets and buccal tablets, USP Apparatus 1 and 2, the basket and the paddle apparatus or mini-basket or mini-paddle apparatuses may be used. Lozenges may be tested with basket or paddle apparatus at high agitation (175 rpm) or with USP Apparatus 3 (reciprocating cylinder apparatus), whereas USP Apparatus 5 (paddle over disk) or a mini-basket may be used for testing films. The use of miniaturized equipment serves to reduce the dissolution or release medium volumes to less than 500 mL, given a smaller fluid volume available in the oral cavity. By contrast, no product performance test is specified for solutions and sprays, presumably because dissolution or release should not in theory be a concern for these solution products.

As shown in Table 5, for a handful of oropharyngeal products, relevant tests are specified in product specific *USP* monographs. In addition, the FDA's Dissolution Methods Database also provides information on dissolution or release test methods for certain drugs formulated in oropharyngeal products. Table 6 summarizes the methodological details of the methods listed therein.

Table 4. Oropharyngeal Drug Products Listed in USP <4> and Current USP Performance Tests According to USP <1004>

Dosage Form, <4>	Performance Test, <1004>
Buccal patches	<724> Apparatus 5
Films	<724> Apparatus 5, Apparatus 1[a]
Gels	<1724>
Gums	European Pharmacopoeia
Lozenges	<711> Apparatus 1[b], Apparatus 2[b], Apparatus 3
Ointments	<1724>
Solutions (rinses)	—
Sprays	—
Tablets (buccal, sublingual)	<711> Apparatus 1[a], Apparatus 2[a]

[a]*Miniaturized version.*
[b]*At high agitation (175 rpm).*

Methodological Standardization

The product-specific *USP* monographs listed in Table 5 stipulate the *USP* disintegration test or dissolution test for eight oropharyngeal drug products as a performance test. However, according to *USP* <1004>, the *USP* disintegration test is not specified as a performance test for buccal and sublingual tablets. By contrast, the methods listed in the FDA's Dissolution Methods Database vary for a given drug or a given dosage form type, and no standardization has been made to date. In line with *USP* <1004>, the basket and the paddle apparatus, and USP Apparatus 5 (paddle over disk) are most frequently suggested, whereas the reciprocating cylinder apparatus is indicated for mini-lozenges of nicotine polacrilex and sublingual tablets

Table 5. Methodological Details of Disintegration or Dissolution Tests for Drugs Formulated in Various Oropharyngeal Drug Products, Found in USP Drug Product Monographs

Dosage Form	Drug	Apparatus	Medium	pH	Volume (mL)	Agitation (rpm)	Duration (min)
Lozenge	Clotrimazole	USP 2[a]	HCl (0.1 N)	1	500	50	45
	Zinc; Vitamin C	USP 2[b]	HCl (0.1 N)	1	900	75	60
Tablet (buccal)	Methyltestosterone	Disintegration[c]	Water	—	—	—	30
Tablet (sublingual)	Buprenorphine and Naloxone	USP 1[a]	Water	—	500	100	10
	Isosorbide dinitrate	USP 2[a]	Water	—	900	50	20
	Isosorbide dinitrate	Disintegration[c]	Water	—	—	—	2
	Nitroglycerin	Disintegration[c]	Water	—	—	—	2
	Ergotamine tartrate	Disintegration[c]	Water	—	—	—	5

[a]*Dissolution <711>.*
[b]*Disintegration and Dissolution of Dietary Supplements <2040>.*
[c]*USP Disintegration <701>, (for uncoated tablets)*
HCL: hydrochloric acid.

Table 6. Methodological Details of Dissolution or Release Performance Tests for Drugs Formulated in Various Oropharyngeal Drug Products, Found in the FDA's Dissolution Methods Database

Dosage Form	Drug[a]	Apparatus	Medium	pH	Volume (mL)	Agitation	Duration
Film (buccal)	Buprenorphine	USP 1 (100 mL)	Phosphate buffer	4.5	60	100 rpm	60 min
	Fentanyl	USP 1 (100 mL)	Phosphate buffer	6.4	60	100rpm	45 min
	Fentanyl	USP 1 (100 mL)	Phosphate buffer	6.5	100	100rpm	45 min
Film (sublingual)	Apomorphine	USP 5	Bis-Tris buffer	6.4	500	75 rpm	20 min
	Buprenorphine and Naloxone	USP 5 (56 mm; 40 mesh disk)	Acetate buffer	4.0	900	100 pm	10 min
Gum	Nicotine	EP	Phosphate buffer	7.4	20	60 cycles/min	30 min
Lozenge	Nicotine	USP 1	Phosphate buffer	7.4	900	100 rpm	8 h
	Nicotine	USP 3	Phosphate buffer	7.4	250	20 rpm	90 min
	Fentanyl	USP 2	Phosphate buffer	4.5	500	175 rpm	40 min
Tablet (buccal)	Acyclovir	USP 1	Phosphate buffer	6.0	1000	60 rpm	12 h
	Miconazole	USP 1	0.5% SDS in water	6.5	1000	60 rpm	12 h
	Fentanyl	USP 2 (small volume)	Phosphate buffer	7.0	100/200	100 rpm	20 h
	Testosterone (ER)	USP 2 (sinker)	0.5% SDS in water	—	1000	60 rpm	24 h
Tablet (sublingual)	Buprenorphine	USP 1	Water	—	500	100 rpm	15 min[b]
	Asenapine	USP 2	Acetate buffer	4.5	500	50 rpm	5 min
	Buprenorphine/Naloxone	USP 2	Water	—	500	100 rpm	20 min
	Fentanyl	USP 2	Phosphate buffer	6.8	500	50 rpm	20 min
	Nitroglycerin	USP 2	Phosphate buffer	6.5	500	50 rpm	10 min
	Zolpidem	USP 2	Phosphate buffer	6.8	900	75 rpm	15 min
	Zolpidem	USP 2	Simulated intestinal fluid	6.8	500	50 rpm	15 min
	Sufentanil	USP 3	Acetate buffer	4.5	50	50 rpm	15 min

[a]Drugs may be in different chemical forms, e.g., salt or polacrilex.
[b]Or until 80% of the labeled content is dissolved.
SDS: Sodium dodecyl sulfate; ER: Extended release.

of sufentanil (Table 6). Both the dissolution or release medium (water, buffer, or simulated body fluid), pH (4.0–7.4), volume (20–1000 mL), and agitation speed (20–170 rpm) vary between test methods. In this regard, the volume of the dissolution or release medium is certainly of particular interest for adaptations, as a volume >500 mL, as described in *USP* <711> and <724> for testing oral dosage forms, is probably of little relevance when trying to represent fluid conditions in the oral cavity. In fact, *USP* <1004> mentions the use of a mini-basket or mini-paddle apparatus to accommodate smaller fluid volumes (e.g., 20–100 mL, as suggested for some products) given the limited volume of oral mucosal fluid in the oral cavity. The duration of the dissolution or release tests also varies. Fast-acting drugs (e.g., sublingual tablets) are tested for 5–20 min, whereas other products are tested for up to 24 h. It is therefore clear that these methodological differences in dissolution or release testing need to be resolved by standardization, presumably according to the type of dosage form.

Biorelevance of In Vitro Test Conditions

Because, as mentioned above, the USP dissolution or drug release apparatuses were developed and validated for testing the performance of other (e.g., oral, or transdermal) dosage forms rather than oropharyngeal drug products, methodological adaptations are required. Accordingly, a lack of biorelevance is evident. The use of more than 500 mL of dissolution or release medium is likely to maintain "sink" conditions in many cases, but this should not necessarily be the case for drugs with low solubility, e.g., lipophilic drugs. Smaller media volumes (e.g., 20–100 mL) can be used with mini-basket and mini-paddle systems. The smaller media volumes may still be larger than the fluid volume available in the oral cavity within a short time for rapidly dissolving or releasing dosage forms, such as sublingual tablets, films, and even lozenges. The composition and pH of the dissolution or release medium should be chosen to reflect the fluid at the site of drug release, i.e., oral mucosal fluid or saliva. For some products, such as buccal tablets, gels, and ER

ointments, drug release should occur only on the surfaces that come into contact with the oral mucosa, and this must be considered during testing. At the same time, for release tests of such dosage forms, no rationale arises in terms of agitation of the medium to simulate the dynamics of oral mucosal fluid. However, there are also dosage forms, such as lozenges, where mechanical stress in particular can have a direct influence on the dissolution behavior, which is why in these cases one should also consider the influence of the mixing of the medium or, for example, the agitation rate in devices such as the reciprocating cylinder apparatus. Finally, the residence time of drugs and dosage forms in the oral cavity is not considered in current compendial test methods. After dissolution or release, dissolved or dispersed drugs may be rapidly excreted from the oral cavity via salivary clearance, and if this is the case, such drugs no longer exert local therapeutic effects or, in the case of systemically active drugs, may no longer be absorbed through the oral mucosa. Meanwhile, a need of biorelevance can even be debatable, especially for rapidly dissolving or releasing dosage forms, if drug dissolution or release anyway occurs in a short period, unaffected by compositions and conditions of surrounding media.

For assessing drug release of gums, *USP* <1004> endorses the official dissolution test for medicated chewing gums (2.9.25) from the *EP*. The two official apparatuses, Apparatus A and B, are both closed chamber systems with horizontal and vertical oscillatory pistons, respectively, to reflect deformation of gums and masticatory actions of subjects. The recommended release medium is 20 mL of phosphate buffer at 37°, as is also found for nicotine gum in the FDA's Dissolution Methods Database (Table 6). Nevertheless, other parameters such as distance between upper and lower chewing surfaces, rotation angle, and chewing frequency, as well as sampling volume and duration still need to be rationally chosen. Indeed, some of them have been shown to affect drug release from gum products (*29*, *30*). In 2015, a *Stimuli* article (*31*) reported a multilaboratory study to test two nicotine gum products on the US market using Apparatus A and B of the *EP*. Despite applying identical procedures and set-ups, variability in the drug release profiles was high and the results for a given product differed between the two apparatuses. These few experiments are certainly not nearly sufficient to identify a method as suitable or unsuitable. In fact, evaluation of new methods requires a much larger number of experiments with different drug products and careful selection and control of experimental conditions, including performance verification tests with reference standards. Hence, *USP* has not yet published its own performance tests for medicated chewing gums.

As stated before, no performance (dissolution or release) test is stipulated for oral spray products. This is presumably because the marketed products deliver an aliquot of drug in solution to a defined location within the oral cavity, e.g., into the mouth over the tongue, and precipitation on the oral mucosal surface would not be expected by virtue of a low dose and a decent aqueous solubility. However, this presumption may or may not be true, if a low solubility drug is formulated or a suspension spray product becomes available in the future. Therefore, even at this point, there should be thought ahead about what the design for a biorelevant and meaningful in vitro performance test for such products could be.

Oropharyngeal drug products are used not only for systemic disease treatments but also for topical local disease treatments. Examples for such topical local treatments are liquids (solutions and suspensions), semisolids (gels, creams, and pastes), chewing gums, and films (patches and strips), to treat oral mucositis, candidiasis, infection, pain relief, or anaesthesia, as well as lozenges to treat sore throat (*32*). However, the current *USP* dissolution or release tests for oropharyngeal drug products (Table 5) may or may not be appropriate to examine local action performance of these products. This is in fact true for many locally acting drug products administered through different routes (e.g., skin, eye, ear, nose, and lung), recognizing that local drug concentrations and profiles are of importance, rather than systemic counterparts. Nevertheless, the dissolution or release profiles may provide some information on the behavior of the products in the oral cavity, whereas the lack of biorelevance and assessment of the residence time of the active ingredient in the current tests may prevent accurate prediction of local drug concentrations and thus performance. Depending on the nature of the locally active dosage form, new methods to be established will certainly not have to meet the same requirements in all cases. For example, testing for some dosage forms, such as locally acting oropharyngeal gels and ointments (*33*), will likely involve use of the vertical diffusion cell for topical and transdermal drugs described in *USP* chapter <1724>. However, it should be noted that there are efforts on the horizon to demonstrate local bioavailability of oropharyngeal dosage forms using appropriate in vitro release testing in generic drug product evaluation. A detailed example of the development of a suitable method for a locally acting lozenge formulation comprising the use of a simulated salivary fluid and an apparatus that mimics fluid exchange in the oral cavity as well as mechanical forces that can act on the dosage form has recently been published (*34*, *35*). The basic considerations for the design

of the cited method can certainly provide a basis for the development of other biorelevant methods, but it should also be clear that the test conditions based on a successful study are not necessarily transferable to all types of lozenges. Here, as with many other oropharyngeal drug products, there is still room to develop standardized, robust, but meaningful performance tests.

In Vitro-In Vivo Correlation

Because current performance tests for oropharyngeal drugs are not biorelevant, IVIVC has not been widely practiced. For systemic applications, challenges are foreseeable for many products when swallowed drugs are absorbed from the gastrointestinal tract, as systemic PK profiles are composed of the fraction of dose absorbed via the oral mucosa and the gastrointestinal mucosa. For example, for a fentanyl buccal lozenge, approximately 25% of the dose is absorbed through the oral mucosa with the remaining 75% being absorbed through the gastrointestinal tract, which is reflected in double peaks in the resulting plasma profiles (*36*). In contrast, for a rapidly dissolving fentanyl buccal tablet, approximately 50% of the administered dose is reported to be absorbed through the buccal mucosa, whereas the remainder is swallowed and absorbed in the gastrointestinal tract (*37*). With this mixed absorption, it is uncertain whether dissolution or release-based performance tests alone are sufficient to predict systemic PK profiles and thus product performance. It may be that drugs are dissolved or released, as predicted by in vitro performance tests, but are readily removed from the oral cavity by salivary clearance, so no local effects are expected. This indicates that in the future it will presumably be difficult to implement standardized methods that allow for an IVIVC for every type of oropharyngeal dosage form.

Vaginal Route

Vaginal drug products are particularly appropriate for drugs associated with women's health issues but may also have applications in general drug delivery within the female population. Whereas historically vaginal drug products have been administered primarily for local effects, for example, to treat infections of bacterial, fungal, or viral origin, or to administer contraceptive or labor-inducing agents, the vaginal route of administration has recently gained more interest because it is also well suited for the administration of a number of drugs with systemic effects (*38*). Systemic administration of drugs via the vaginal mucosa can have several advantages. These include the avoidance of multiple side effects that may result from oral or parenteral administration of the corresponding drugs, but especially the bypass of hepatic first-pass metabolism (*39*).

The vagina represents a slightly S-shaped muscular canal of about 10 cm in length. This canal is collapsed so that the anterior and posterior vaginal walls are in contact (*40*). The vaginal wall as such is about 3 mm thick and consists of three layers. The uppermost layer, i.e., the vaginal epithelium consists of stratified, nonkeratinized squamous epithelial cells, the thickness of which is subject to constant change related to the female menstrual cycle (*41*). The inner mucosa of the vagina also has numerous folds called rugae that provide extensibility, support, and increase the surface area of the vaginal wall. Under the squamous epithelium lies a very elastic layer of connective tissue (lamina propria), permeated by veins, due to which the vagina has excellent elasticity, allowing sexual intercourse and childbirth, but also the administration of vaginal dosage forms.

Similar to the thickness of the vaginal epithelium, the amount and composition of vaginal fluid changes during the menstrual cycle and with age. Estrogen and sexual stimulation increase the secretion of vaginal fluid (*38*). Although women of reproductive age produce 3–4 g of vaginal fluid per hour, this decreases to about half after menopause (*41*). Vaginal fluid as such does not exist; rather, it is a mixture of different secretions, such as cervical secretions and mucus, endometrial and oviductal fluid, transudate from blood vessels containing exfoliated vaginal cells and leukocytes, and microorganisms and their metabolites (*38*, *40*, *41*). Accordingly, it contains a variety of components, such as inorganic and organic salts, mucins, proteins, carbohydrates, urea, and fatty acids (lactic and acetic acids) (*38*). The pH conditions in the vagina are determined by the bacterial flora present. Under normal conditions, i.e., vaginal eubiosis, lactobacilli convert glycogen from exfoliated cells into lactic acid, thus maintaining a buffered acidic environment in the pH range of about 3.5–4.5 (*38*, *40*–*42*). During menstruation, but also due to frequent sexual intercourse, an increase in vaginal pH can be recorded because both ejaculate and vaginal transudate are alkaline. Moreover, vaginal dysbiosis (e.g., bacterial vaginosis) can also lead to noticeable changes in vaginal pH (*41*, *43*). Overall, the parameters that can affect intravaginal drug release or dissolution are quite complex, which may place special demands on the in vitro performance tests to be established, especially when it comes to predicting performance under typical application conditions. A variety of vaginal dosage forms are currently on the market or in clinical development. To date, in the United

States, 145 vaginal drug (48 reference listed drugs (RLD) and 97 generic drug products) products of which 71 have been discontinued have been approved for clinical use (FDA *Orange Book*).

Performance Tests

According to *USP* <4>, vaginal drug products are classified into creams, foams, gels, and inserts. As shown in Table 7, for performance testing of vaginal gels, reference is made to the methods for determining drug release from semisolid dosage forms described in the existing *USP* <1724>. However, because the test procedures in this general chapter were developed and validated for semisolid formulations for cutaneous application rather than for vaginal drug products, appropriate methodological adaptations are required, such as those described in *USP* <1092>. The same applies to vaginal inserts, for which reference is made to *USP* <711> and the use of the basket or the paddle apparatus, which are devices originally developed for dissolution testing of oral dosage forms. For foams, *USP* <1004> does not specify performance tests aimed at investigating the release of the active ingredient, presumably because these are preparations in which the active ingredient is dissolved, and the release of the active ingredient is ensured by the nature of the preparation.

Table 7. Vaginal Drug Products Listed in USP <4> and Current USP Performance Tests According to USP <1004>

Dosage Form, <4>	Performance Test, <1004>
Creams	<1724>
Foams	—
Gels	<1724>
Inserts	<711> Apparatus 1, Apparatus 2

Although the *USP* contains several individual monographs for vaginal drug products (six vaginal creams, two vaginal suppositories, and six vaginal inserts), only one of these monographs, that for *Estradiol Vaginal Inserts*, describes a product-specific drug release test, which must be performed in 500 mL of phosphate buffer, pH 4.75 in the basket apparatus over a test period of 10 h. For all other vaginal inserts, a disintegration test according to *USP* Disintegration <701> is required instead of a dissolution test (Table 8). Interestingly, however, <701> does not contain specific information on how to determine the disintegration of vaginal inserts, so consideration should be given to modifying the method at this point if necessary.

The FDA's Dissolution Methods Database contains several individual dissolution or drug release test methods for certain drugs formulated into vaginal drug products. Table 9 summarizes the methodological details for each. Although this database does not include in vitro performance tests for semisolid preparations for vaginal application, it does include test methods for vaginal inserts and tablets, which are referred to as inserts in the *USP*. In addition, it lists methods for dosage forms such as vaginal rings and vaginal suppositories that are not listed in *USP* chapters <4> and <1004> and also indicates the need to develop in vitro methods to characterize in vitro release of these two dosage form types.

Methodological Standardization

The dissolution method in the *USP* for estradiol inserts (Table 8) as well as most of the FDA-approved test methods (Table 9) specify the use of compendial equipment, such as the basket or the paddle apparatus or a slightly modified basket apparatus (Palmieri basket [*44*] in combination with relatively large volumes (500–900 mL) of aqueous media with varying pH (4.5–7.4) and composition (water, hydrochloric acid, phosphate buffer). In cases where the formulation contains poorly soluble drugs, artificial surfactants such as sodium dodecyl sulfate (SDS) are added to the medium to provide sink conditions. Agitation in the basket or paddle set-up ranges from 40 and 100 rpm and the test duration varies between 30 min and 12 h. For one of the dosage forms, a vaginal ring containing estradiol, a noncompendial in vitro set-up, i.e., an incubator shaker, is used. In this set-up, the vaginal ring is immersed in 250 mL of 0.9% saline solution at pH 6.5 and agitated at 130 rpm for a test duration of 45 days. For selected drug products, such as a dinoprostone suppository or an ethinylestradiol and etonogestrel ring, for which a suitable in vitro performance test does not yet

Table 8. Methodological Details of Disintegration or Dissolution Tests for Drugs Formulated in Various Vaginal Drug Products, Found in USP Drug Product Monographs

Dosage Form	Drug	Apparatus	Medium	pH	Volume (mL)	Agitation (rpm)	Duration
Insert	Nystatin	Disintegration[a]	Water	—	—	—	60 min
	Clotrimazole	Disintegration[a]	—	—	—	—	20 min
	Estradiol	USP 1	Phosphate buffer	4.75	500	40	10 h

[a]*<701>, Procedure and Criteria for Uncoated or Plain-Coated Tablets.*

Table 9. Methodological Details of Dissolution or Release Performance Tests for Drugs Formulated in Various Vaginal Drug Products, Found in the FDA's Dissolution Methods Database

Dosage Form	Drug	Apparatus	Medium	pH	Volume (mL)	Agitation	Duration
Insert	Dinoprostone (ER)	USP 2	Deionized water	4.5	500	50 rpm	5 h
	Progesterone	USP 2	0.25% SDS in water	6.4	900	50 rpm	30 min
Ring	Estradiol	Incubator shaker	0.9% Saline	6.5	250	130 rpm	45 days
Suppository	Miconazole nitrate	USP 1	0.45% SDS in water	7.4	900	100 rpm	8 h
	Terconazole	USP 1[a]	1% SDS in 0.1 N HCl	4.5	500	100 rpm	40 min
Tablet	Estradiol	USP 1	Phosphate buffer	4.75	500	40 rpm	12 h
	Clotrimazole	USP 2	0.1 N HCl	—	900	50 rpm	45 min

[a]*With a Palmieri type basket.*
ER: Extended release; SDS: sodium dodecyl sulfate; HCL: hydrochloric acid.

exist, the database specifically notes the need to develop an appropriate method for in vitro release testing.

As for oropharyngeal drug products, adapting the volume of the dissolution or release medium is certainly of interest when aiming method standardization as a volume >500 mL might be of little relevance when trying to represent fluid conditions in the vagina. However, before entering too deeply into such a discussion, it should be borne in mind that vaginal dosage forms are also a very heterogeneous group of dosage forms that are used either for local or systemic action and range from dosage forms with very rapid release up to those with sustained release of the active ingredient over a period of weeks or even months. Therefore, as for oropharyngeal drug products, it is not possible at this point to attempt a generally applicable method standardization. One must rather distinguish whether the active ingredient is predominantly delivered into the vaginal lumen, or immediately in the vicinity of the mucosa, whether it is intended for local or systemic action, and whether it is released over minutes, hours, days, weeks, or months. In the end, this distinction will not only determine the volume and composition of the medium, but also a whole range of other analytical parameters. In addition, in the context of considering the standardization of test methods for vaginal drug products, an additional aspect emerges that should definitely be taken into account. Whereas *USP* <4> distinguishes vaginal dosage forms into creams, foams, gels, and inserts (Table 7), both the FDA Dissolution Methods Database and the *Orange Book* refer to dosage forms such as vaginal tablets, rings, ER inserts, and suppositories. Consequently, the question arises as to whether these formulations can be considered subsets of the vaginal drug products listed in *USP* <4>, or otherwise how to deal with these formulations. Especially when it comes to developing an appropriate and standardized performance test, answering this question would be of vast importance. According to *USP* general chapter <1151>, inserts are referred to as solid dosage forms that are inserted into a naturally occurring body cavity other than the mouth or rectum. They can be applied for local or systemic action. Vaginal inserts are described as globular or oviform dosage forms that are intended to dissolve in vaginal secretions. Although the description of vaginal inserts indicates that these are preparations that dissolve in the vaginal fluid, contradictory information follows in the next section, which deals with possible manufacturing processes for inserts (not limited to vaginal inserts). Here, it is stated that the inserts may be molded, pressed from powder or, in the case of extemporaneous formulations, even formulated as capsules, and that they may be formulated so that they melt at body temperature or disintegrate on insertion. These explanations do not necessarily contribute to great clarity. This is why, on the one hand, it would be reasonable to include vaginal tablets and suppositories in the category of vaginal inserts because they are inserted into the vagina and dissolve, melt, or disintegrate there. On the other hand, especially if one considers the composition and the characteristics of the different dosage forms (hydrophilic, lipophilic), they could be considered individual types of dosage forms for which different performance tests will be required. Moreover, it should be noted that ER inserts and vaginal rings do not appear anywhere in the *USP*. Before considering standardization of performance tests for vaginal drug products, thought should be given to standardizing the terminology and clearly distinguishing between the individual dosage forms. In the next step, a suitable method could then be selected depending on the type of dosage form, mode of action (systemic or local), intended drug release time, drug properties, and dose. For hydrophilic formulations containing highly soluble drugs, miniaturized standard methods, as already discussed for oropharyngeal drug products, would certainly be suitable. For lipophilic suppositories, suitable

methods could for instance be established based on EP chapter 2.9.42, *Dissolution Test for Lipophilic Solid Dosage Forms*.

For vaginal rings, there is currently no official method describing a release test using a compendial set-up. The release method for an estradiol ring described in the FDA Dissolution Methods Database is representative of many incubator shaker-based methods reported in the literature (*45*). Such methods are often used to compare different prototypes in the development of vaginal rings. An incubation shaker can be equipped with a variety of vessels so that many rings can be tested in parallel. In addition, the set-up is easy to handle. Both these features are advantageous if the test duration is weeks or even months. In terms of standardization, however, such a method must be viewed rather critically, which is why there is also a need for suitable performance tests here. Because vaginal rings, as already mentioned, are usually intended to release the active ingredient over several weeks or even months, if one wishes to standardize test methods, a completely different question arises at the same time, namely the applicability of accelerated test methods. Based on the results of some studies on the acceleration of drug release without influencing the release mechanism of a vaginal ring (*46*, *47*), the use of such methods as in vitro performance tests seems generally possible, which, however, requires a very precise control of all test conditions as well as an appropriate method validation.

Biorelevance of In Vitro Test Conditions

If one considers the currently described in vitro release methods for vaginal drug products and assesses them with regard to their biorelevance, a similar picture emerges at many points as for oropharyngeal drug products. The site of application, i.e., the vagina, is also a body cavity with a small amount of liquid available and, in contrast to the oral cavity, a much lower fluid exchange. In vitro drug release methods using several hundred milliliters of fluid can therefore hardly reflect the physiological conditions in the vagina, but at best create sink conditions for poorly soluble drugs. As for oropharyngeal drug products, for rapidly dissolving or releasing dosage forms, such as hydrophilic inserts, suppositories, and tablets, smaller media volumes can be used with mini-basket and mini-paddle systems, yet the fluid volume again may still be larger than the fluid volume available in the vagina.

In recent years, especially because it has been shown that microbicides applied topically to the vagina by women can reduce the risk of infection with HIV and other sexually transmitted diseases, there has been a trend towards the development of novel vaginal dosage forms (*48*, *49*). This led with an increasing demand for appropriate in vitro test methods for ensuring a safe and reliable in vivo performance of these novel formulations. Accordingly, there have been several attempts to make release methods more biorelevant, as evidenced in particular by the introduction of various simulated vaginal fluids (*50*). This was a first step toward establishing more biorelevant IVRT methods for these dosage forms. However, the instrumental set-ups used are usually conventional as discussed before and the methods presented so far have generally been used to compare specific formulations in individual experiments. Overall, methods that could be claimed to be biorelevant or even biopredictive and capable of becoming generalizable are still lacking (*51*).

In Vitro-In Vivo Correlation

To date, few efforts have been reported to establish an IVIVC for a vaginal dosage form. Given the lack of physiology-based biorelevant release models for vaginal dosage forms to date, this is not surprising. It should be mentioned, however, that it has recently been possible to retrospectively correlate the mean in vivo release rate of a contraceptive vaginal ring with its in vitro release performance. This was achieved both with a real time release method, in which a medium with a physiologically relevant pH was used, as well as with various accelerated test methods, in which the temperature and/or the medium composition was specifically changed to accelerate drug release (*46*, *47*). Results of the respective studies demonstrate that it is possible to obtain in vivo predictive results for vaginal preparations using appropriate in vitro methods. However, due to fundamentally different release mechanisms and the dependence on the test conditions used, the method developed for the vaginal ring in question cannot simply be transferred to other vaginal dosage forms. Therefore, further substantial work is needed when aiming to predict in vivo performance of vaginal drug products based on in vitro performance testing.

Rectal Route

The rectum represents the last section of the colon and opens into the anal canal. In adults, it has a length of about 10–15 cm and a diameter like that of the sigmoid colon. The anal canal itself has a length of 3–4 cm. The rectum and anal canal have a special sphincter system, which ensures continence and also defecation (*52*). The rectum is normally empty and the anal canal is closed by permanent contraction of the internal sphincter (*53*, *54*). A specific pattern of contraction of the empty rectum

prevents continuous outflow of colonic contents into the rectum, and therefore fecal matter remains in the sigmoid colon until it is ready to be excreted from the body (*54*). When feces pass from there into the rectum, the rectal wall stretches, and the internal anal sphincter relaxes to accommodate the feces. A certain amount of rectal stretching eventually triggers an urge to defecate so that controlled defecation can occur. Even though the rectum can store up to 2 L of stool in the interim, as noted earlier, it is usually empty most of the time, and the open diameter of the rectal lumen is then no more than 1.5–3.5 cm. Histologically, the rectum shows similarities to other sections of the colon. The mucosa has a smooth surface with a total surface area of approximately 200–400 cm^2 (*55*, *56*) and consists of simple squamous enterocytes with straight tubular glands that run through the entire thickness of the mucosa. The rectum is drained by three veins: the inferior, middle, and superior rectal vein. The inferior and middle rectal vein empty into the systemic venous system, thus avoiding a hepatic first-pass effect of rectally administered drugs absorbed via these veins. In contrast, the superior rectal vein opens into the portal venous system. For this reason, complete avoidance of hepatic first-pass metabolism cannot be guaranteed with rectal administration of a drug. In this context, one should also consider that anastomoses between the portal and systemic veins may be present in the wall of the anal canal. Unfortunately, there is very little information on available volumes, secretion rates, composition, and properties of rectal fluid. Secondary literature reports that the adult rectum is "filled" with 1–3 mL of a nearly enzyme-free, viscous fluid with a pH in the neutral to slightly alkaline range (7.2–8) and virtually no buffering capacity (*4*, *57*, *58*). Moreover, there is evidence that age and diet may influence rectal pH. Unfortunately, however, robust data on these statements are not available, and further studies are needed to better understand the rectal environment in different patient groups.

Rectal drug products can be used for both local and systemic administration. Due to its size, the rectal lumen can accommodate relatively large dosage forms. Consequently, high drug doses can be administered rectally. However, although the ability to administer large dosage forms and high doses of a drug provide excellent conditions for controlled-release drug delivery systems, the use of such dosage forms is essentially precluded because drug delivery can be interrupted or terminated at any time by defecation. Therefore, rectal administration is typically used for immediate-release (IR) dosage forms (*59*). In the United States, 145 rectal drug products of which 108 are generic drug products have been approved for clinical use. To date, 83 of these drug products have been discontinued (FDA *Orange Book*).

Performance Tests

USP <4> classifies rectal dosage forms into foams, ointments, suppositories, solutions, and suspensions (Table 10). For performance testing of ointments, reference is made to the methods for determining drug release from semisolid dosage forms described in the existing general chapter <1724>. However, because the test procedures in this general chapter were developed and validated for semisolid formulations for cutaneous application rather than for rectal drug products, appropriate methodological adaptations are required, such as those described in *USP* general chapter <1092>. The same applies to suppositories, for which reference is made to <711> and the use of the basket or the paddle apparatus, the flow-through cell (USP Apparatus 4), and in particular, a modified, dual-chamber flow-through cell also described in chapter 2.9.42. of the EP that prevents analytical interferences caused by oil droplets formed during the melting of lipophilic suppositories. As for vaginal foams and all other solutions for mucosal administration, *USP* general chapter <1004> does not specify performance tests aimed at investigating drug release of rectal foams and solutions, as these are considered preparations in which the active ingredient is dissolved, and the release of the active ingredient is ensured by the nature of the preparation. For drug release testing of suspensions, the basket or the paddle apparatus or a miniaturized basket or paddle system can be used. As already discussed for other mucosal drug products, the use of miniaturized equipment serves to reduce the dissolution or release medium, but not to an extent that would be required to mimic physiological fluid volumes in the rectum.

Table 10. Rectal Drug Products Listed in USP <4> and Current USP Performance Tests According to USP <1004>

Dosage Form, <4>	Performance Test, <1004>
Foams	—
Ointments	<1724>
Suppositories	<711> Apparatus 1, Apparatus 2, Apparatus 4[a]
Solutions	—
Suspensions	<711> Apparatus 2b

[a]*Standard set-up or flow-through cell designed for suppositories.*
[b]*Standard or miniaturized version.*

The *USP* contains a single product-specific monograph (*Indomethacin Suppositories*), that describes a product-specific drug release for a suppository. In that case, the experiment is to be performed in the paddle apparatus in

900 mL of phosphate buffer at pH 7.2 over a test period of 60 min. Three dissolution methods for rectal drug products, i.e., a gel, a suspension, and a suppository can be found in the FDA's Dissolution Methods Database (Table 11). Furthermore, the need to develop an in vitro method for another suppository formulation is expressed.

Methodological Standardization

Although rectal drug products listed in the FDA's Dissolution Methods Database (Table 11) are basically different dosage forms with quite distinct release mechanisms, the paddle or basket apparatus in combination with large liquid volumes (500–900 mL) are to be used for the IVRTs. This is particularly surprising for studying drug release from a gel. While in two cases a phosphate buffer (pH 6.8 or 7.2) has to be used, the release experiment for prochlorperazine suppositories is to be performed in 0.1 N hydrochloric acid, a medium whose composition and pH in no respect correspond to the rectal fluid. It is also interesting to note that instead of the usual basket, a so-called Palmieri basket, named after its developer, representing a suppository basket made of inert plastic with the same dimensions as the standard basket, but in which the meshes have been replaced by 12 linear slits, must be used. By contrast, the indomethacin suppositories monographed in *USP* have to be tested for in the paddle apparatus in 900 mL of phosphate buffer at pH 7.2. The agitation rates in paddle (50 rpm) and basket system (100 rpm) represent standard agitation speeds and the test durations range from 45 and 60 min indicating that all dosage forms represent IR formulations.

As for oropharyngeal and vaginal drug products, a volume of >500 mL is unlikely to be relevant when trying to represent fluid conditions in the rectum. When aiming to standardize test conditions, one should certainly consider an adaptation of the media volume to more physiological volumes. As indicated in <1004>, mini-basket or mini-paddle devices may be suitable to address this issue. Because all rectal dosage forms are rapid-release dosage forms, it should be fairly easy to standardize test durations. As quite reliable pH conditions prevail in the rectum, ranging from neutral to slightly alkaline, a release medium should reflect this if possible. Accordingly, a medium such as 0.1 N hydrochloric acid should not appear in a test method for rectal dosage forms unless there are special reasons for allowing only such a medium. Whereas such a procedure should be appropriate for rectal suspensions, when aiming to standardize IVRT conditions for suppositories one should first refer to <1004>, which distinguishes two types of suppositories: 1) hydrophilic (water soluble), and 2) lipophilic (oil soluble or melting). Whereas drug release (dissolution) of water-soluble suppositories can be studied using the (mini) paddle or basket apparatus or the flow-through cell, as discussed earlier, drug release testing for lipophilic suppositories may need modification of the dissolution procedure to avoid analytical interference from the oil globules. As for lipophilic vaginal suppositories, a suitable method could be established based on *EP* chapter 2.9.42, *Dissolution Test for Lipophilic Solid Dosage Forms*, taking into account the aspects already mentioned (*60*).

Biorelevance of In Vitro Test Conditions

Consideration of the biorelevance of current in vitro release methods for rectal drugs gives a picture similar to that already discussed for a number of other mucosal dosage forms. Like the vagina, the rectum is an application site with a low fluid supply and a very low rate of fluid exchange. Therefore, current IVRT methods that require the application of large volumes of fluid provide, at best, sink conditions for poorly soluble drugs, but they cannot be considered biorelevant. Even with a miniaturized test method, one will hardly be able to match real fluid volumes. Overall, there seems to be quite little activity in the area of in vitro release testing of rectal dosage forms. In contrast to many other mucosal application areas, where there have been at least initial attempts to develop biorelevant media for in vitro testing of various kinds, no simulated or artificial rectal fluid has yet been reported. Therefore, there is a need not only for standardization of test methods, but at the same time also for increasing the biorelevance of IVRTs of rectal dosage forms.

Table 11. Methodological Details of Dissolution or Release Performance Tests for Drugs Formulated in Various Rectal Drug Products, Found in the FDA's Dissolution Methods Database

Dosage Form	Drug	Apparatus	Medium	pH	Volume (mL)	Agitation (rpm)	Duration (min)
Suspension	Mesalamine	USP 2	Phosphate buffer	7.2	900	50	30
Gel	Diazepam	USP 2	0.05 M Phosphate buffer	6.8	500	50	45
Suppository	Prochlorperazine	USP 1[a]	0.1 N HCl	—	900	100	45

[a]*With a Palmieri type basket.*
HCL: Hydrochloric acid.

In Vitro-In Vivo Correlation

To date, no biorelevant IVRT methods have been developed that aim to predict the in vivo performance of rectal drug products and there have been no efforts to correlate data from standard release studies with in vivo data. Accordingly, fundamental work is needed if it is to become possible to establish IVIVC based on results from in vitro performance tests.

Urethral Route

Urethral drug products comprise dosage forms that are inserted into the urethra, typically for local action, but systemic distribution of the administered drugs is also possible.

The urethra is a tubular organ of the urinary and genital apparatus. Due to its close association with the genital organs, which are differentiated by gender, the urethra is also distinct in the genders. It begins at the lower end of the urinary bladder localized in the pelvis and opens at the tip of the penis on the glans in males and in the vaginal vestibule in females. The anatomy and function of the urethra differ significantly in males and females. The male urethra is about 20 cm long and due to its incorporation into the penis has two curvatures as well as three constrictions in its progression. It serves not only to drain urine, but also as a canal for prostatic secretions and semen. The female urethra, on the other hand, is straight and only 3–5 cm long, and its function is limited to the discharge of urine from the bladder (*61*). The average open diameter (~6–8 mm) and the wall structure of some sections of the male and female urethra are similar. The urethral wall consists of three layers: Like all urinary drainage pathways, it has a special lining called the urothelium or transitional epithelium. When the urethra is empty, this lining is raised into longitudinal folds (*61*). Beneath the epithelium are elastic connective tissue and a blood vessel plexus. This is followed further out by smooth muscle and, on the very outside, again by connective tissue, which anchors the urethra in the surrounding tissue. In both men and women, the ducts of various glands open into the lumen of the urethra. Unfortunately, there is little information in the literature about the amount and the composition of fluid present and the pH conditions in the urethra. Overall, however, it can be assumed that the intraluminal pH conditions are dominated by the pH value of the urine (normal range: pH 4.5–7.8) or, in men in the event of ejaculation, also by the pH value of the seminal fluid (normal range: pH 7.2–8.0) (*62*).

To date, a medicated dissolvable urethral suppository, which according to the USP nomenclature would be referred to as an insert, is the only intraurethral drug product approved by the FDA. This suppository contains alprostadil and is inserted into the urethra immediately after urination. The drug is intended to show a fast action by local diffusion into the body tissues to initiate arteriolar vasodilation and penile erection.

Certainly, the application of drugs in the urethra is a very special field of application for which probably only a limited number of dosage forms can be expected in the future. Nonetheless, it should be noted that new therapeutic options have recently emerged for this field of application as well, such as the use of paclitaxel-coated balloon catheters for the treatment of urethral strictures (*63*). For these reasons, it is important to have suitable in vitro performance tests available for the quality assurance of such formulations. To date, eight urethral drug products can be found in the *Orange Book*, five of these are solutions for which a performance test in the sense of a drug release test is generally not required, and three of these are urethral suppositories.

Performance Tests

According to <4>, there is currently only one category of urethral drug products, namely, urethral inserts and no performance tests are described. Like the USP, the FDA's Dissolution Methods Database does not contain a monograph for a urethral drug product. Likewise, no alternative performance test has been described in the literature so far. At this point, it should be noted that there are currently only three suppository formulations on the market, which are alprostadil formulations of various potencies that are inserted into the male urethra in the form of a pellet or rod, referred to as a suppository, immediately after urination and before sexual intercourse. The aim of this procedure is to achieve rapid release and absorption of the active ingredient in order to achieve an erection. For this dosage form, it would first be necessary to develop a performance test, whereby the question arises as to whether this should then also be suitable for new dosage forms for urethral application or whether a universal test method can be developed for urethral drug products in general.

Methodological Standardization

Currently, no official performance tests for urethral drug products are available and general recommendations for their development do not exist. Therefore, it is reasonable to assume that in the case of the development of a new product, if required, a product-specific performance test will be developed. If in the future, several new drug products will be developed, it is likely that, as has happened in the past for other mucosal dosage forms,

different individual methods will be published rather than a standardized method being developed. The latter, however, should be the goal, as all urethral drug products are applied to a site characterized by a narrow lumen, usually direct contact with the tissue and with a small volume of fluid present. Because there are as yet no methods that would have to be considered in the further course of the decision, it would be desirable to develop a biorelevant test method from the very beginning, which would allow an estimation of the in vivo performance, but which would also be robust and simple enough to be used in quality control.

Biorelevance of In Vitro Test Methods

As already discussed, neither standard release methods nor biorelevant in vitro test methods for urethral drug products currently exist. In general, no considerations for the development of biorelevant test methods for these dosage forms can be found in the current scientific literature. As the urethra is normally flushed several times a day during urination, it seems unlikely at first glance that sustained release formulations would be considered for this site of application, because urination could cause washout released drug or the entire drug product and the amount of drug reaching the target site could not be controlled. For IR formulations, one could consider developing an in vitro model comprising a release medium that addresses the average urine pH for both male and female applications. A distinction would have to be made, in general, between dosage forms for systemic and for local action, especially when it comes to establishing sink conditions. Taking into account the apparatus described in *USP* chapters <711> and <724>, the development of a standardized method based on an official *USP* apparatus, such as the flow-through cell, is quite conceivable. Depending on the dosage form under investigation, such a method could probably also be further adapted to suit particular physiological conditions, such as for instance a certain disease state that presents with alterations of urethral fluid pH and/or composition, by varying the release medium and other test parameters if the aim is to increase in vivo predictivity. Since, as already described, there is currently only one commercially available drug product and there are yet little further research approaches towards new dosage forms for this site of application, it would be speculative to discuss further methodological details at this point.

In Vitro-In Vivo Correlation

As there are currently no release methods for urethral dosage forms, the question of an IVIVC does not (yet) arise.

GENERAL DISCUSSION AND CONCLUSION

Mucosal drug products represent a very heterogeneous group of dosage forms that are applied to various sites of the body. The formulations can differ significantly in their formulation design and release properties, whereas the characteristics of the application site, i.e., the various mucosae and other conditions at the site of application, can also differ greatly. Accordingly, it quickly becomes apparent that there can be no universal recommendation for suitable performance tests for all of these dosage forms, but that—as has been done in the previous sections—one must take a closer look at essential characteristics of the dosage form and the application site, but also to take into account whether a local or a systemic effect is to be achieved, to define suitable test conditions. A review of the currently available compendial test methods for mucosal drug products indicates that these considerations were probably not present in the first place during method selection and development. Thus, for many of the dosage forms in question, one finds either no methods at all, or in *USP* general chapter <1004>, reference to standard methods available in *USP* chapters <711>, <724>, <1724>, or <1771> is made, in most cases without addressing the fact that many of the test conditions listed there are unlikely to reflect conditions at the application site and thus may not provide the best basis for developing a meaningful performance test. A closer look at official test methods for individual drug products also reveals a frequent use of standard test methods whose test design often cannot be reconciled with the mode and site of application. This is not to question the suitability of these methods for the quality control of the respective drug products, but it does indicate that it is time to reconsider how meaningful performance tests could be developed and standardized. Results of the gap analysis indicate that, especially in view of evidence that the number of mucosal dosage forms is likely to increase in the future and that more application fields will be identified, it is necessary to update several of the existing methods and to introduce new performance tests for the various subtypes of mucosal drug products.

The need to develop new performance tests that are biorelevant and predictive where possible has also been recognized elsewhere. For example, under the Generic Drug User Fee Amendment (GDUFA), the FDA has funded various research programs to establish equivalence standards for complex pharmaceutical drug products, including several mucosal drug products. Among the stated research objectives here were the development of novel IVRTs that more closely match physiological in vivo conditions, the development of physicochemical

characterization methods to evaluate and compare critical quality attributes of various mucosal products and to determine key physicochemical properties that affect drug release and bioavailability, as well as further development of in silico modeling to study the effects of formulation properties on PK and/or pharmacodynamics (PD).

Some possibilities for the development of biorelevant and predictive performance tests have already been discussed in the individual subsections of this article. However, this *Stimuli* article is not intended to provide specific guidance for each individual subcategory of mucosal drug products, but rather to initiate a discussion among experts aimed at developing appropriate methods, balancing biorelevance, predictivity, robustness, and standardization as best as possible. A whole range of critical aspects and challenges relevant to method development including the choice of the apparatus, the volume, composition, physicochemical properties and temperature of the test medium, agitation or flow rate, that would also need to be considered here have already been discussed in a previous *Stimuli* article of this series (*5*). Although many general aspects apply to all dosage forms, it should be emphasized once again for the mucosal drug products that the dosage form is not permanently surrounded by liquid at any application site, that the available liquid volumes are generally small, but can differ significantly again at the application sites and that, in addition, the condition of the mucous membranes also varies. In addition, as already mentioned, a distinction must be made between dosage forms for local and systemic action.

Biorelevant media have already been proposed for almost all mucosal application sites (*64*, *65*, *50*). These differ considerably in composition and physicochemical properties, as is particularly evident from the example of the simulated vaginal fluids (*50*). A first important step in the direction of meaningful performance tests is certainly to first address the standardization of media for the individual application sites. The desired goal should be a medium that reflects the parameters relevant for drug release at the application site as optimally as possible, can be produced easily and reproducibly and, in combination with other test parameters, results in a robust and discriminatory method that is meaningful without being too complex. The next step would be to consider whether and when it would be appropriate to modify official test methods for certain dosage forms in such a way that they permit the use of smaller media volumes. Due to the diversity of mucosal dosage forms, one would have to consider at the same time whether special hydrodynamic conditions are required and whether and how sink conditions could be guaranteed. In this context, a distinction must certainly be made as to whether the dosage form is to adhere to the mucosa over the entire application time or whether it can or should move freely in the body cavity concerned and whether a unidirectional or multidirectional release is to be achieved. Diffusion methods appear to be particularly useful for dosage forms that are applied to the mucosa to deliver an active ingredient into the systemic circulation. Here, the question of suitable diffusion membranes arises. If the aim is to establish a robust test method, it would seem sensible to work with artificial diffusion membranes, which, however, are not necessarily suitable for mucoadhesive preparations because they probably cannot reproduce the interaction of the preparation and the mucosa that occurs in vivo. For the latter formulations, one would accordingly have to think about biomimetic membranes, cell models, or even natural membranes, although with increasing complexity, standardization of methods will become increasingly difficult. Dialysis-based release methods, in which the dosage form together with a small volume of biorelevant fluid is placed into the lumen of a dialysis tube that is agitated in a larger volume of a suitable acceptor fluid, might represent an interesting approach for the development of performance tests of dosage forms that are applied into body cavities where they can be wetted by or immersed in small amounts of fluid (*23–25*, *66*, *67*).

Because an ideal IVRT method should correlate the changes in the critical quality attributes of the drug product that have directly related to release performance, as has already been suggested or practiced for various types of semisolid drug products, including ophthalmic ointments (68), typical physicochemical properties of the pharmaceutical product in question that have the potential to affect product performance should be correlated with release behavior as part of method development and validation in order to develop meaningful, discriminatory, and robust test methods.

This article was written to raise awareness of challenges in standardizing drug release test methods for mucosal drug products. The points discussed here are intended to provide a starting point for future activities in the area of performance test development and for readers to provide food for thought for a fundamental discussion on this topic. It is our expressed wish that this *Stimuli* article will encourage a collaborative effort to reconsider, and if necessary, revise current methods, as well as to fill currently existing methodological gaps with standardized

and, in terms of in vivo performance, predictive test methods where possible. The EP-NAPPT Subcommittee will therefore greatly appreciate the involvement of as many stakeholders as possible in the activities of the EP-NAPPT by providing comments and suggestions based on their experience, so that the review and revision process now initiated can be jointly pursued further and future activities can move in the right direction.

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dx.doi.org/10.14227/DT300423P224

Comparative Dissolution of Budesonide from Four Commercially Available Products for Oral Administration: Implications for Interchangeablity

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ABSTRACT

Budesonide is a corticosteroid regularly used in oral formulations to treat various inflammatory diseases in the gastrointestinal tract, such as Crohn's disease and ulcerative colitis. Budesonide has also been formulated to be effective against immunoglobulin A (IgA)-related nephropathy. This study aimed to compare the release of budesonide from four oral formulations under discriminating dissolution conditions to ascertain if they are interchangeable. Each product had a unique dissolution profile along with differences in indications, doses, and dosing conditions. As such, there is no basis for considering the tested products to be pharmaceutically or therapeutically interchangeable.

KEYWORDS: Budesonide, oral products, dissolution, release profile, interchangeability

INTRODUCTION

Budesonide is a corticosteroid regularly used in oral formulations to treat various inflammatory diseases in the gastrointestinal (GI) tract, such as Crohn's disease and ulcerative colitis. Because these inflammatory conditions affect different parts of the GI tract, different formulations have been introduced over the years to deliver budesonide selectively to inflamed tissue.

Entocort EC is indicated for treatment of active mild to moderate Crohn's disease at a daily dose of 9 mg (three 3-mg capsules once daily) or 6 mg (two 3-mg capsules in the morning) to maintain patients in remission (*1*). The equivalent European product, Entocort has similar indications, with the addition of microscopic colitis (*2*). Although Entocort is approved for treatment of the disease in both the ileum and ascending colon, the capsules consist of beads with an enteric coating (Eudragit L55, Evonik GmbH, Germany) designed to dissolve at pH 5.5 and above, so budesonide release likely starts proximally in the small intestine. Once the enteric coating on the beads is dissolved, an ethylcellulose component provides sustained release of budesonide.

Budenofalk (currently marketed in Europe but not available in the USA) is indicated for induction of remission in patients with mild to moderate active Crohn's disease affecting the ileum and/or the ascending colon at a daily dose of 9 mg (either three 3-mg capsules in the morning or one capsule in the morning, one at midday, and one in the evening). Budenofalk has also been approved for the treatment of microscopic colitis and autoimmune hepatitis (*3*). Like Entocort EC, the oral formulation consists of enterically coated beads housed in a capsule, but the coating is a mixture of Eudragit L and Eudragit S. The combination of Eudragit L and S in the coating formulation has a higher nominal pH of release (pH 6.4) than that of Eudragit L alone, so budesonide release from Budenofalk likely begins more distally in the small intestine than from Entocort EC (*4*). Once the enteric coating components on the bead dissolve, prolonged release of the active component is provided by Eudragit RS.

Cortiment 9-mg prolonged-release tablets are approved for induction of remission in patients with mild to moderate active ulcerative colitis (UC) in cases where 5-aminosalicylic acid (ASA) treatment is not sufficient, as well as to induce remission in adult patients with active microscopic colitis. Tablets are taken with or without food in the morning, taking care not to break, crush, or chew the tablet, because the film coating is intended to ensure a prolonged release (*5*). However, because the

coating is a mixture of Eudragit L and S with a release pH of 7, it serves more as a delaying rather than a prolonging component. Prolonged (extended) release of budesonide is ensured by embedding the drug in a multimatrix (MMX) formulation. The applications of MMX formulations have been described (*6*).

Nefecon (TARPEYO in US; Kinpeygo in UK) 4-mg delayed release capsules are another oral product containing budesonide. Unlike the other commercially available formulations, Nefecon is indicated to reduce urine protein levels in adults with primary immunoglobulin A (IgA) nephropathy who are at risk of rapid disease progression (*7*). Nefecon is formulated to target the release of budesonide to gut-associated lymphoid tissue, in particular the Peyer's patches (*8*). Four capsules are taken in the morning 1 hour before food intake. Instead of enterically coated beads, the capsule itself is coated. The beads containing budesonide are housed in the capsule, and release from the beads is regulated by an ethylcellulose-based coating.

The aim of this study was to compare release of budesonide from these four products under discriminating dissolution conditions to ascertain if they are interchangeable in terms of delivering budesonide to the same location within the GI tract.

METHODS

Materials

Four commercially available, orally administered, delayed-release budesonide products were compared with respect to their release characteristics:

- Entocort (Batch #32448, Tillot's Pharma)
- Budenofalk (Batch #L21017A, Dr. Falk Pharma)
- Cortiment (Batch #P152, Ferring Pharma)
- Nefecon/Tarpeyo (Batch #3193032, Calliditas Therapeutics)

The pharmaceutical characteristics of these products are summarized in Table 1.

All materials used for ultra high-performance liquid chromatography (UHPLC) analysis were of analytical grade.

Dissolution Studies

The release of budesonide from the four commercial products was compared using the same USP apparatus 2 (paddle) dissolution tester (VK 7000, VanKel) under two sets of experimental conditions.

In the first set of experiments, the products were studied according to *United States Pharmacopeia* (USP) general chapter <711> Dissolution, following the two-stage experimental design (method B) for delayed-release oral products, but with 900 mL per vessel (instead of 1000 mL) and $n = 3$ (*9*). Media were prepared according to *USP* directions for simulated gastric fluid (SGF) and simulated intestinal fluid (SIF), which has a buffer capacity of 30 mmol/L/pH unit.

In the second set of experiments, a biorelevant method with reduced buffer capacity in the intestinal phase was used to capture the essential aspects of release from enteric-coated products in vivo. Each product was subjected to an acidic environment (SGF, pH 1.2) for 2 hours to represent the maximum gastric residence time of the product when administered on a fasted stomach, followed by exposure to an almost neutral environment (pH 6.5) to represent pH conditions in the small intestine. Media were prepared with fasted-state simulated intestinal fluid version 1 (FaSSIF v1) buffer concentrate from Biorelevant.com (London, UK). After dilution according to the manufacturer's instructions, this buffer had a pH of 6.5 and a capacity of 12 mmol/L/pH unit (*10*). The tests with the biorelevant method were conducted with $n = 6$.

Enteric coatings are polymeric acids, so dissolution is highly dependent on both pH and buffer capacity of the

Table 1. Pharmaceutical Characteristics of Delayed-Release Budesonide Oral Formulations

Parameter	Nefecon	Budenofalk	Entocort	Cortiment
Enteric coating material and component	Eudragit L and S on capsule shell	Eudragit L and S on beads	Eudragit L55 on beads	Eudragit L55 and S on tablet
Nominal pH of enteric coating	Proprietary information[a]	pH 6.4 (RMS Assessment Report)	pH 5.5 (FDA)	pH 7 (FDA)
Capsule material	HPMC	Gelatin	Gelatin	N/A
Sustained-release component	Ethylcellulose-based coating on beads	Eudragit RS	Ethylcellulose	MMX (stearic acid/HPC matrix)

[a]*Nominal pH is between that of Entocort and Budenofalk (written communication, Calliditas Therapeutics).*
RMS: Regulatory Management System of the Medicines and Healthcare Products Regulatory Agency (MHRA), UK; HPMC: hydroxypropyl methylcellulose; MMX: multimatrix formulation; HPC: hydroxyproplycellulose.

dissolution media. Ozturk et al. demonstrated that when the buffer capacity at a given pH is reduced, the acidic enteric coating counteracts buffer ions approaching the coating surface more effectively, thus maintaining lower pH at the dissolving surface of the coating and decreasing the rate of dissolution (*11*). Likewise, Markopoulos, Andreas et al. reported that pH and buffer capacity are the two key factors required to achieve biorelevance in dissolution testing of products with enteric coatings (*10*). Therefore, in the intestinal phase of the test, the concentration of the buffer species (buffer capacity) was lowered to correspond more closely to the in vivo environment in the small intestine.

For all dissolution studies, the media volume was 900 mL, the paddle speed was 100 rpm, and the temperature was 37 + 0.5 °C. Samples (10 mL) were filtered through 25-mm Whatman filters (6890-2507 GD/X). The first 8 mL of filtrate was discarded, and the last 2 mL were retained for UHPLC analysis.

Ultra High-Performance Liquid Chromatography (UHPLC) Analysis

Analysis of all dissolution samples was conducted using a Vanquish UHPLC system (Dionex Softron GmbH, Germering, Germany), including a binary pump H (VH-P10-A) powered by Smart Flow, fitted with an Acquity UPLC BEH C18 column (1.7 µm, 2.1 × 50 mm). The mobile phase solution consisted of 2.70 g sodium acetate trihydrate in 1 L water in a ratio of 420:580 (v/v) with acetonitrile. The operating temperature was 40 °C. These conditions resulted in a run time of 1 minute, with elution of budesonide as a single peak between 0.46 and 0.53 minutes.

The lowest concentration used for the calibration curve was 40 ng/mL, corresponding to 1% dissolution from a 4-mg capsule. The correlation coefficient for the calibration curve was 0.999 or higher in each run. Mean recovery of budesonide was 99% for standard solutions in gastric media and 102% in phosphate buffer.

The method development for this analytical procedure is described in laboratory journal 21E2197 (RI.SE AB, Sweden).

Statistical Analysis

Dissolution results are presented as mean percent of drug release with standard deviation. The data are also presented in tabular form in the Supplementary Material (available online). The f_2 statistic (similarity factor) was calculated using Nefecon (Tarpeyo) as the reference product to compare with the other products. Statistics were calculated with Microsoft Excel.

RESULTS

USP Method

Drug release profiles using the general USP methodology are shown in Figure 1. None of the products released an appreciable amount of budesonide in the acid (gastric) phase of the experiment (pH 1.2 for 2 hours), so only results in the buffer (intestinal) phase are shown.

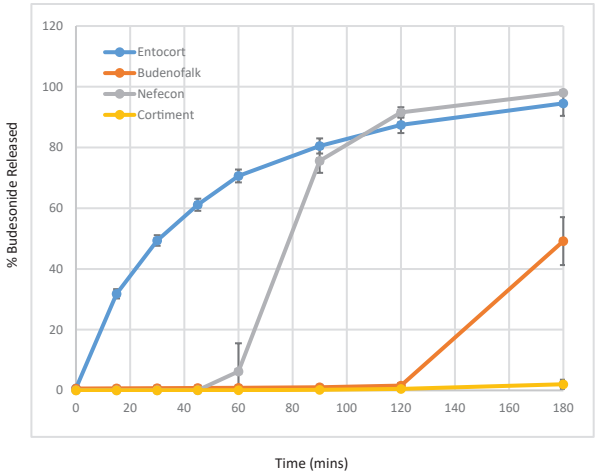

Figure 1. Dissolution profiles (mean ± SD % release) of four delayed-release budesonide oral formulations using the USP <711> method B. Only the buffer (intestinal) phase is shown; no appreciable release of budesonide was observed in the acid (gastric) phase.

The four products displayed clearly different dissolution profiles under the USP test conditions. As expected from its Eudragit L55 coating (nominal pH of release is pH 5.5), the Entocort product started to release budesonide immediately in the pH 6.8 dissolution medium, then budesonide release was sustained over approximately 3 hours. By contrast, the Cortiment product released a minimal amount of budesonide within the 3-hour test period, reflecting a higher nominal pH of 7.0 for initiation of release, which, together with the MMX used to sustain release after the enteric coating dissolves, minimizes release at pH 6.8. Budenofalk, with a nominal pH of 6.4 for initiation of release, started to release after 2 hours of exposure to the pH 6.8 medium. The sustained-release component (Eudragit RS) in Budenofalk limited release to approximately 50% between the 2- and 3-hour mark. Nefecon had yet another distinct dissolution profile, with little or no release in the first 60 minutes of exposure to the pH 6.8 dissolution medium, followed by a modestly sustained-release pattern, with most of the release occurring during the second hour of the intestinal phase of the test.

Biorelevant Method with Reduced Buffer Capacity

Drug release profiles in the FaSSIF v1 buffer (intestinal phase) are shown in Figure 2.

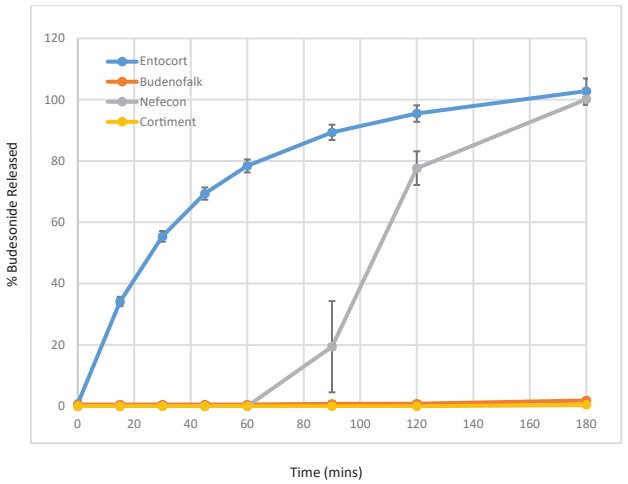

Figure 2. Dissolution profiles (mean ± SD % release) of four delayed-release budesonide oral formulations using the biorelevant method with reduced buffer phase. Only the buffer (intestinal) phase is shown; no appreciable release of budesonide was observed in the acid (gastric) phase.

Large differences in budesonide dissolution profiles were observed among the four products. For Budenofalk, the release was more delayed more under biorelevant conditions compared with *USP* <711> conditions, most likely because the pH of the dissolution medium was adjusted to pH 6.5, which is close to the nominal pH of release from this product. With lower buffer capacity in the dissolution medium, the pH at the surface of the enteric coating is expected to remain lower, curbing the onset of release (*11*).

Table 2 shows the f_2 values for comparison of Nefecon with the other products. An f_2 value of 50 or greater is required to demonstrate similarity of the profiles (*12, 13*). In line with the visual inspection of the release profiles, all f_2 values failed to meet this criterion for similarity by a very wide margin.

Table 2. Similarity Factor (f_2) Analysis for Comparison of Dissolution Profiles

	Nefecon	Entocort EC	Budenofalk	Cortiment
f_2 value (USP)	Reference	18.1	16.0	15.8
f_2 value (biorelevant)	Reference	11.7	16.1	15.8

DISCUSSION

The selection of dissolution test conditions for these studies was based on *USP*, FDA, and EMA guidelines. The first round of tests was run essentially under USP <711> Method B conditions for oral delayed release products, the only variations being a dissolution volume of 900 mL rather than 1000 mL and a more granular sampling schedule. With a pH of 6.8, these conditions conform to one of the conditions in the FDA's Product Specific Guidances (PSG) for delayed-release budesonide products (*14–16*). The PSGs call for dissolution at several pH values ranging from 4.5 to 7.5. As the aim of the study was to discriminate among the formulations with respect to onset and rate of budesonide release, dissolution at pH 4.5 (at which no release is expected because pH < pKa of the Eudragit coatings) and pH 7.2 and 7.5 (at which 100% release is expected because pH > pKa of the Eudragit coatings) was not studied. Instead, the focus was on pH values that are most likely to be discriminating for enteric coatings, i.e., pH 6.5 and 6.8. The EMA guideline on studies for demonstrating therapeutic equivalence for locally applied, locally acting oral products suggests investigating the effect of various physiological factors on release, including buffer strength and intraluminal pH (*17*). Therefore, the experimental conditions used in this study were based on these guidances combined with considerations from Ozturk et al. and Markopoulos, Andreas et al. (*10, 11*).

Under both experimental conditions, the release profile of budesonide differed widely among the four commercial products. None of the f_2 comparisons demonstrated similarity. Thus, the release profiles for the tested products are considered strongly dissimilar and not pharmaceutically equivalent.

The Nefecon release profile in biorelevant conditions showed that 1) capsule disintegration and onset of release are delayed until approximately 1 hour after switching from the acid (gastric) phase to the FaSSIF v1 buffer (intestinal) phase, 2) the majority of drug release subsequently occurs within 2 hours, and 3) complete budesonide release is reached within 3 hours in the intestinal phase. The comparatively short period of release under intestinal conditions is expected to result in a more localized release compared with the other products. Since the passage time through the small intestine is typically 3.5–4.5 hours, the Nefecon release pattern is commensurate with the stated target of Nefecon therapy, which is to deliver budesonide selectively and specifically to the ileum (*18–20*).

By comparison, Entocort starts releasing budesonide almost immediately upon exposure to intestinal conditions, irrespective of the buffer conditions. This can be attributed to the formulation with Eudragit L55, an enteric coating that starts to release at a comparatively low pH of 5.5. Thereafter, release is sustained over a period of about 3 hours, with almost 80% released in the first hour of exposure to intestinal conditions. As a result,

it is expected that most of the budesonide in Entocort is delivered to more proximal regions of the small intestine.

In contrast to Entocort, Budenofalk only releases budesonide at small intestinal pH under highly buffered (*USP*) conditions. Furthermore, the release starts after 2 hours of exposure to these conditions, consistent with Budenofalk's therapeutic objective of releasing budesonide to the distal ileum and the caecum, whereby a substantial amount of budesonide is delivered into the colon (*2*). Differences in release profiles for Entocort® and Budenofalk have been previously reported by Klein et al. (*21*).

Cortiment failed to release significant amounts of budesonide under either lightly or highly buffered conditions at intestinal pH levels, which is in line with its target of releasing budesonide to the colon to treat ulcerative colitis (*5*).

When considering interchangeability among these budesonide products, one must also consider further aspects of their characteristics, such as the indications for which they are approved, the dose strengths available, and the recommended dosing conditions. Of the four products, only Nefecon specifies that the dosage form must be taken 1 hour before food in the morning. The other three products have no limitation on food intake, and for Budenofalk there is the possibility to split the dosing up over the day. Given that Cortiment contains 9 mg of budesonide, the only product that could be substituted from a dosage point of view is Budenofalk. The prescriber information for Entocort specifies two 3-mg capsules per day (i.e., maximum of 6 mg) for maintenance therapy or three 3-mg capsules per day for active inflammation, while Nefecon capsules contain 4 mg of budesonide, making it impossible to match the dose of any of the other three products, except at 12 mg, a dose that is not approved for any of other products. Uniquely, and because of the higher dose (four 4-mg capsules per day), dose tapering is additionally recommended when discontinuing therapy with Nefecon.

Finally, the indications for which the products are approved differ substantially. Entocort and Budenofalk are both approved for the treatment of mild to moderate Crohn's disease and to maintain patients with this disease in remission. Budenofalk is also approved for the treatment of microscopic colitis and autoimmune hepatitis. Unlike the anti-inflammatory indications for Entocort and Budenofalk, Cortiment is approved for the induction of remission in patients with mild to moderate active UC if 5-ASA treatment is not sufficient. Cortiment is also approved to induce remission in adults with active microscopic colitis, but (unlike Budenofalk) it is not approved for autoimmune hepatitis. Nefecon is the only product of the four that is approved to reduce urine protein levels in adults with primary IgA nephropathy who are at risk of rapid disease progression.

Because the doses, dosing conditions, approved indications, and dissolution profiles of the four budesonide products studied differ so widely, substituting one of these products for another cannot be either scientifically or medically justified.

CONCLUSION

The dissolution data reported herein support the therapeutic goals of each product tested (Entocort EC for Crohn's disease, Budenofalk for Crohn's and UC, Cortiment for UC, and Nefecon for IgA nephropathy). Given the substantial differences in drug release patterns, widely divergent therapeutic aims, and differences in doses and dosing conditions of the four products, they cannot be regarded as either pharmaceutically or therapeutically interchangeable.

ACKNOWLEDGEMENT

The author thanks RI.SE AB (Sweden) for assistance with the dissolution studies and Dr. Edmund Kostewicz (Fraunhofer ITMP) for statistical analysis.

FUNDING

This study was funded by Calliditas Therapeutics (Sweden).

CONFLICT OF INTEREST

The author disclosed no conflict of interest related to this article.

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dx.doi.org/10.14227/DT300423P230

Dissolution Performance of Verapamil-HCl Tablets Using USP Apparatus 2 and 4: Prediction of In Vivo Plasma Profiles

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ABSTRACT

This study aimed to examine the in vitro dissolution performance of verapamil-HCl reference tablets using USP apparatus 2 (paddle), apparatus 4 (flow-through cell), and media of physiological relevance, and to estimate plasma levels using a convolution approach. The study used apparatus 2 at 50 rpm and apparatus 4 at 16 mL/min. Solutions of 0.1 N HCl, acetate buffer (pH 4.5), and phosphate buffer (pH 6.8) were used as dissolution media. UV quantification of the drug at 278 and 300 nm was registered after 30 min of dissolution. In vitro release was evaluated by model-independent and model-dependent methods. Mean dissolution time (MDT), dissolution efficiency (DE), $t_{50\%}$, and $t_{63.2\%}$ were calculated and statistically compared. The percent of dissolved drug was fitted with first-order, Korsmeyer-Peppas, Makoid-Banakar, Weibull, Logistic, and Gompertz models; Makoid-Banakar and Gompertz were the best-fit equations used to explain drug release. In vivo performance of verapamil-HCl tablets was predicted using apparatus 4 to mimic in vivo plasma concentrations of the drug in humans.

KEYWORDS: Convolution, flow-through cell, USP apparatus 2, USP apparatus 4, verapamil-HCl, dissolution

INTRODUCTION

Verapamil is a derivative of papaverine, belonging to a class of medications known as calcium channel blockers (*1*). It is prescribed to treat arrhythmia and high blood pressure and to control angina pectoris (*2*). Verapamil works by dilating blood vessels, increasing blood flow and oxygenation to the heart, and decreasing the electrical activity of the heart to control heart rate (*3*). It is a highly absorbed drug, with more than 90% of an orally administered dose absorbed (*4*). Despite this, its bioavailability is only 20–30% due to its extensive first-pass metabolism (*5*). It is also a highly variable drug; its apparent volume of distribution is about 2.5 L/kg, peak plasma concentrations are reached within 1–2 h following oral administration, and it has a relatively short half-life (2.8–5 h) (*5–7*).

Information about verapamil-HCl solubility, permeability, pKa, pharmacokinetic properties, and other property data has been collected, but discrepancies exist (*7*). Verapamil-HCl has been classified as a class I drug, but some authors have placed it on a list of compounds with inconclusive data (class I/II drug) (*4, 8*). High (82–11 mg/mL) or low (0.44–0.025 mg/mL) solubility has also been reported depending on the degree of acidity of the surrounding environment (*7*).

The official dissolution test for verapamil-HCl tablets is described in the *United States Pharmacopoeia* (USP) (*9*). The method indicates the use of USP apparatus 2 (paddle) at 50 rpm with 900 mL of 0.01 N HCl at 37.0 ± 0.5 °C as dissolution medium (Q not less than 75% at 30 min). To date, no in vitro/in vivo correlation (IVIVC) has been reported that considers pharmacopeial conditions. On the other hand, the biowaiver monograph for verapamil-HCl tablets provides guidelines for carrying out in vitro studies that allow in vivo studies to be avoided (*7*).

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To suggest a biowaiver based on the Biopharmaceutics Classification System (BSC) for class I drugs, several conditions must be fulfilled; in particular, the drug (test and reference) must rapidly dissolve and the product must not contain any excipient that will affect the rate or extent of absorption (*10*). An immediate-release product is considered rapidly dissolving when a mean of 85% or more of the drug dissolves within 30 min when using apparatus 2 at 50 rpm in 900 mL at pH 1.2, 4.5, and 6.8 (*10*).

USP apparatus 1 (basket) and apparatus 2 (paddle) are currently the most popular methods to carry out dissolution studies. Both apparatus operate under closed finite sink conditions; however, they cannot mimic the hydrodynamic environment of the gastrointestinal (GI) tract (*11*). Because of its characteristics, USP apparatus 4 (flow-through cell) is better suited to estimate the in vivo performance of certain formulations (*12, 13*). Comparative in vitro dissolution studies of verapamil-HCl tablets using apparatus 4 vs. apparatus 1 or 2 are scarce.

This study aimed to evaluate the in vitro dissolution of verapamil-HCl reference tablets under the hydrodynamic environments generated by apparatus 2 and 4 at pH 1.2 (0.1 N HCl), pH 4.5 (acetate buffer), and pH 6.8 (phosphate buffer). Additionally, the dissolution results will be used to predict in vivo plasma profiles of verapamil-HCl utilizing a convolution strategy to assess which method is most promising for mimicking the in vivo performance of verapamil in humans.

MATERIALS AND METHODS

Reagents and Chemicals

Verapamil-HCl tablets (Dilacoran 40 mg, Abbott Laboratories, Mexico City, Mexico) were used. Mexican health authorities have established this commercial brand as the reference product for dissolution and bioequivalence studies (*14*). HCl, CH_3OH, CH_3COONa, H_2PO_4- and HPO_4^{2}- were acquired from J. T. Baker-Mexico (Xalostoc, Mexico). Verapamil-HCl standard was acquired from Sigma-Aldrich Co. (St. Louis, MO, USA).

Absorption Spectrum

To verify the maximum dissolution of verapamil-HCl in pH 1.2 (0.1 N HCl), pH 4.5 (acetate buffer), and pH 6.8 (phosphate buffer), a drug solution of 50 µg/mL was prepared in each medium, and an ultraviolet (UV) spectrophotometer (Lambda 35, Perkin Elmer, USA) was used to measure the absorbance at 200–350 nm.

Validation

Linearity

According to ICH guidelines, three standard curves of verapamil-HCl were prepared in each solution (0.1 N HCl, pH 4.5 acetate buffer, and pH 6.8 phosphate buffer) (*15*). From a methanol stock solution (1 mg/mL), standard solutions of 10, 30, 50, 75, and 100 µg/mL of the drug were prepared at each pH. Absorbances measured at 278 and 300 nm were recorded, and differences in absorbance (278–300 nm) were fitted vs. drug concentration by linear regression. Absorbance differences at these wavelengths have been suggested by the *USP* 42 (*9*).

Accuracy and Precision

Twenty tablets were accurately weighed and crushed in a mortar. Powdered verapamil-HCl tablets were added to a quantity of verapamil-HCl standard (10 mg) to give the equivalent of 80, 100, and 120% of the dose, which was dissolved in 900 mL of 0.1 N HCl acid at 37.0 ± 0.5 °C. USP Apparatus 2 was used at 50 rpm to dissolve the drug. After 30 min, the amount of verapamil-HCl dissolved in each vessel (n = 3) was calculated with the standard calibration curve in 0.1 N HCl. The relative error (%RE) was used as a measure of accuracy and the relative standard deviation (%RSD) was used as a measure of precision.

Solution Stability

Solution stability was evaluated using two solutions of verapamil-HCl in 0.1 N HCl (20 and 80 µg/mL). The solutions were analyzed at 0 h at 25 °C and 24 and 48 h after storage at either 4 or 25 °C. At each temperature after 24 and 48 h, the absolute difference (%AD) was calculated.

Uniformity of Dosage Units and Assay

Uniformity of dosage units and assay were performed according to the procedures described in the *USP* 42 (*9*).

Dissolution Profiles

Dissolution profiles of verapamil-HCl tablets were obtained using a paddle apparatus 2 (Sotax AT7-Smart, Sotax AG, Switzerland) at 50 rpm with 900 mL of 0.1 N HCl, pH 4.5 acetate buffer, or pH 6.8 phosphate buffer at 37.0 ± 0.5 °C (n = 6). Additionally, verapamil-HCl tablets were tested with a flow-through cell apparatus 4 (Sotax CE6, Sotax AG) at a flow rate of 16 mL/min using 22.6 mm cells (internal diameter). Laminar flow was used. Dissolution samples were taken at 5, 10, 15, 20, and 30 mins using glass fiber filters (1.0 µm, Millipore). The amount of verapamil-HCl dissolved was determined by UV spectrophotometry at 278 and 300 nm.

Data Analysis

Dissolution profiles of verapamil-HCl in apparatus 2 vs 4 were evaluated using different comparison methods. For model-independent comparisons, the in vitro release at 30 min (Q_{30}), the area under the cumulative dissolution

curve (AUCC), percent of dissolution efficiency (%DE), and mean dissolution time (MDT) were calculated and statistically compared with a student's t-test. Significant differences were defined as $p < 0.05$. For model-dependent comparisons, in vitro results were adjusted to the hyperbola model. With α and b parameters, $t_{50\%}$ and $t_{63.2\%}$ values were obtained. Dissolution data were adjusted to different mathematical equations (first-order, Korsmeyer-Peppas, Makoid-Banakar, Weibull, logistic, and Gompertz). The model with the highest determination coefficient (R_2) and the lowest Akaike information criterion (AIC) was chosen as the best-fit model (*16*). Data analysis was carried out using the Excel add-in, DDSolver program (*17*).

Prediction of In Vivo Plasma Profiles

In vitro dissolution data can be manipulated to predict the in vivo behavior of verapamil-HCl in humans through a simple numerical convolution method created by Qureshi using an MS Excel spreadsheet (*18*). The method uses reported pharmacokinetic parameters of verapamil such as bioavailability factor (F), elimination rate constant (k_e), and volume of distribution (V_d) to construct plasma drug concentration-time profiles (*7, 19*). Using this, pharmacokinetic parameters such as peak concentration (C_{max}), time to reach peak concentration (T_{max}), and area under the concentration-time curve from zero to infinity ($AUC_{0-\infty}$) were predicted and calculated from profiles by a non-compartmental method (*20*). Detailed calculations were performed as detailed below (*18*).

The in vitro dissolution profile was divided into separate parts where the amount of drug (mg) dissolved within each sampling interval was estimated (X = drug dissolved/strength × 100). After that, the latter was corrected for F, and the observed amount of drug in blood was calculated ($X_{corrected}$ = amount of drug [mg] released within sampling interval × F). Finally, blood concentrations (ng/mL) equivalent to the total amount of verapamil-HCl in blood at different times after ingestion of a tablet were calculated using Equation 1.

$$\frac{Total\ amount\ of\ drug\ in\ blood\ after\ absorption\ at\ time\ t}{V_d \times body\ weight} \times 100 \qquad \text{Eq. (1)}$$

The reported data for the concentration-time profile and pharmacokinetic parameters of the reference drug product Isoptin (80-mg verapamil-HCl) were used to establish the predictability of the convolution method, which was established by the calculation of the mean absolute percent of prediction error (%PE) for C_{max} and $AUC_{0\rightarrow\infty}$ according to Equation 2 (where %PE should not exceed 15%) (*19, 21–23*).

$$\%PE = \left(\frac{observed - predicted}{observed}\right) \times 100 \qquad \text{Eq. (2)}$$

RESULTS AND DISCUSSION

Ultraviolet Spectra

The UV spectra of verapamil-HCl dissolved in 0.1 N HCl, pH 4.5 acetate buffer, and pH 6.8 phosphate buffer were very similar, with maximum measurement found at 278 nm in all cases; at 300 nm, a very small measurement was found (almost zero).

Validation

Linearity

The equations of standard solutions of verapamil-HCl in 0.1 N HCl, pH 4.5 acetate buffer, and pH 6.8 phosphate buffer were $y = 0.0111x + 0.019$ ($R^2 = 0.9997$, $p < 0.05$), $y = 0.0115x - 0.0038$ ($R^2 = 0.9996$, $p < 0.05$), and $y = 0.0119x + 0.005$ ($R^2 = 0.9999$, $p < 0.05$), respectively.

Accuracy and Precision

After 3 days of experiments, the %RSD was found to range from 0.36% to 0.91%, and the %RE was lower than 1.3%.

Solution Stability

At 4 °C and 25 °C, the %AD values were less than 0.86% after 24 h and 48 h of storage, suggesting good stability of verapamil-HCl in solution under all tested storage conditions.

Uniformity of Dosage Units and Assay

Verapamil-HCl tablets were within USP limits. The average ± %RSD of 10 verapamil-HCl tablets in uniformity of dosage unit tests was 101.04 ± 2.31% (85–115% is the USP limit); in assay test with three samples the result was 99.81 ± 0.22% (90–110% being the USP limit) (*9*).

Dissolution Profiles

Dissolution profiles of verapamil-HCl tablets are shown in Figure 1, and profile comparisons are given in Table 1. Verapamil-HCl tablets were more than 85% dissolved within 15 min using both dissolution apparatuses when 0.1 N HCl was used as the dissolution medium. This indicates a very rapid in vitro release of the drug at pH 1.2 regardless of the apparatus used; however, use of the flow-through cell (apparatus 4) affected the rate and extent of verapamil-HCl dissolution, as the drug dissolved considerably slower. When the paddle method (apparatus 2) was used, MDT, $t_{50\%}$, and $t_{63.2\%}$ were significantly lower compared with the flow-through cell ($p < 0.05$); the extent of drug dissolution, represented by Q_{30} and AUCC, was also significantly less ($p < 0.05$). For apparatus 4, Q_{30} and

AUCC values were 89.45% and 2285.0%·min, respectively, compared to 94.14% and 2339.9%·min with apparatus 2, respectively; additionally, the overall DE was slightly lower in the flow-through cell compared to the paddle apparatus ($p < 0.05$).

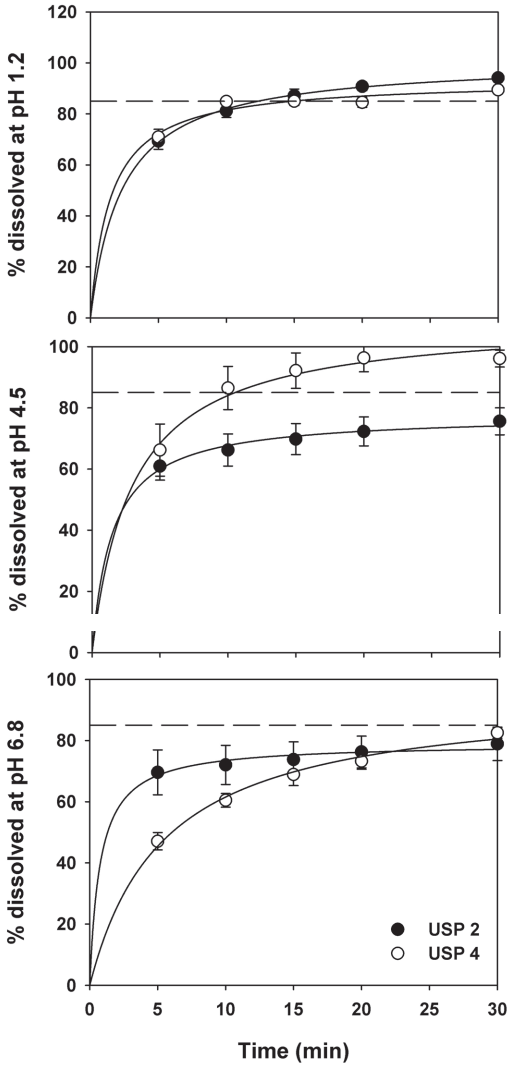

Figure 1. Dissolution profiles of verapamil-HCl tablets (mean ± SD, n = 6). Dashed line shows 85% of dissolved drug.

Drug dissolution in pH 4.5 acetate buffer was faster when using the flow-through cell compared to the paddle. The reference tablets released 96.12% of drug within 30 min compared to 75.6% with the paddle method ($p < 0.05$). This was further confirmed by AUCC values (Table 1). The rate of drug dissolution was faster for apparatus 4 as indicated by $t_{63.2\%}$ at 4:12 min:sec vs 7:10 for apparatus 2. A significantly higher overall DE was found with apparatus 4 at pH 4.5 compared to apparatus 2.

Given the limited solubility of verapamil-HCl at higher pH, dissolution testing at pH 6.8 was more discriminative, as the solubility of the drug is only 11 mg/mL (*7*). Release of verapamil-HCl from reference tablets was less than 85% at 30 mins in both dissolution apparatus; only 78.93% and 82.53% of the drug dissolved with apparatus 2 and 4, respectively. The flow-through cell method resulted in significantly slower dissolution of verapamil-HCl compared to the paddle method.

Overall, significant differences in dissolution parameters were found beyond MDT and $t_{50\%}$ at pH 4.5 and Q_{30} at pH 6.8. At least 85% of the drug dissolved within 15 min in both dissolution apparatuses at pH 1.2, but only with the apparatus 4 at pH 4.5. When pH 6.8 phosphate buffer was used, less than 85% of drug was released at 30 mins in both apparatuses. Therefore, verapamil-HCl reference tablets do not meet the biowaiver criterion established for class I drugs. The dissolution rate of verapamil-HCl was lowered by increased pH in the flow-through cell, whereas the opposite was true in the paddle apparatus; this may be attributed to the different hydrodynamic conditions generated by each apparatus.

Table 1. Dissolution Parameters of Verapamil-HCl Tablets

pH	Parameter	USP Apparatus 2	USP Apparatus 4
1.2	Q_{30}	++	++
	AUCC (%·min)	2339.9 ± 19.0	2285.0 ± 12.5*
	DE (%)	78.00 ± 0.63	76.17 ± 0.42*
	MDT (min:sec)	5:08 ± 0:12	4:27 ± 0:06*
	$t_{50\%}$ (min:sec)	2:17 ± 0:09	1:40 ± 0:08*
	$t_{63.2\%}$ (min:sec)	3:540 ± 0:15	3:02 ± 0:13*
4.5	Q_{30}	-	++
	AUCC (%·min)	1904.6 ± 52.2	2427.1 ± 52.0*
	DE (%)	63.49 ± 1.74	80.91 ± 1.73*
	MDT (min:sec)	4:48 ± 0:10	4:43 ± 0:42
	$t_{50\%}$ (min:sec)	2:45 ± 0:16	2:36 ± 0:24
	$t_{63.2\%}$ (min:sec)	7:10 ± 1:06	4:12 ± 0:37*
6.8	Q_{30}	-	+
	AUCC (%·min)	2043.6 ± 64.1	1844.1 ± 19.2*
	DE (%)	68.12 ± 2.13	61.47 ± 0.64*
	MDT (min:sec)	4:06 ± 0:24	7:39 ± 0:12*
	$t_{50\%}$ (min:sec)	1:26 ± 0:22	6:03 ± 0:12*
	$t_{63.2\%}$ (min:sec)	3:32 ± 1:01	10:50 ± 0:21*

*Values are shown as the mean value ± standard error medium, n = 6. *: Significant difference ($p < 0.05$) compared to apparatus 2; ++: at least 85% dissolved within 15 min (very rapidly dissolving); +: at least 80% dissolved within 30 min; -: less than 80% dissolved within 30 min.*

Results of the adjustment to the mathematical models are shown in Table 2. The data were well-fit to the Makoid-Banakar model using apparatus 2 at all pH levels and using apparatus 4 at pH 4.5 and 6.8. To compare dissolution profiles at pH 4.5 and pH 6.8 with a model-dependent approach, a student's t-test was carried out with kMB

and k parameters; when this was performed, differences were found ($p < 0.05$). At pH 1.2, the dissolution data of apparatus 2 and 4 were best fitted with the Makoid-Banakar and Gompertz models, respectively. As different mathematical equations explained the in vitro dissolution performance of verapamil-HCl at pH 1.2, no comparison was made.

From the obtained results, the dissolution behavior of verapamil-HCl differs between the paddle method and flow-through cell method; however, the different hydrodynamic environments that each piece of equipment generates over the solid dosage means that these differences were expected. To identify the apparatus that generates the most accurate data, the MDT (as a model-independent parameter) and $t_{63.2\%}$ (as a model-dependent parameter) were plotted for each dissolution apparatus. Both parameters represent the time at which the same extent of verapamil-HCl dissolves (Fig. 2).

Only data obtained with apparatus 4 gave a significant linear regression ($p < 0.05$). MDT and $t_{63.2\%}$ obtained by different methods maintained linearity only with data produced by apparatus 4. This indicated that the in vitro dissolution performance of verapamil-HCl tablets in apparatus 4 was more accurate than the dissolution behavior in apparatus 2, regardless of the dissolution media pH (pH 1.2–6.8).

Some authors have studied the effect of the hydrodynamic environment surrounding solid dosage forms. Wu et al. studied the rate underlying tablet dissolution to better understand the role of external hydrodynamic conditions on mass transfer rate and film thickness during in vitro dissolution tests (*24*). Gao explained that apparatus 1 and

Table 2. Results of Dissolution Data Adjustment.

Apparatus	pH	First-Order		Korsmeyer-Peppas		Weibull		Logistic		Gompertz		Makoid-Banakar				
		R^2	AIC	R^2	AIC	R^2	AIC	R^2	AIC	R^2	AIC	R^2	AIC	k_{MB}	n	k
2	1.2	0.9700	12.52	0.9456	16.20	0.9907	6.79	0.9864	8.26	0.9829	9.99	0.9940	2.38	46.19	0.28	0.01
	4.5	0.9405	6.68	0.9718	7.71	0.9722	6.39	0.9691	5.04	0.9661	4.92	0.9936	-4.09	49.95	0.12	4.16×10^{-4}
	6.8	0.9243	6.23	0.9302	4.75	0.9164	5.59	0.9067	6.07	0.9024	6.27	0.9645	2.84	62.25	0.03	-2.71×10^{-3}
4	1.2	0.6319	23.78	0.7081	22.56	0.7695	21.22	0.8007	20.36	0.8095	20.09	0.7020	22.27	52.48	0.23	0.01
	4.5	0.9458	14.95	0.7214	26.44	0.9333	15.49	0.9400	15.93	0.9389	16.57	0.9751	13.94	33.93*	0.49*	7.61×10^{-3}*
	6.8	0.9662	16.45	0.9685	15.16	0.9742	16.04	0.9656	16.11	0.9566	16.49	0.9826	12.55	26.89*	0.38*	5.20×10^{-3}*

*Mean value, n = 6. *: Significant difference compared to apparatus 2 ($p < 0.05$). AIC: Akaike information criterion.*

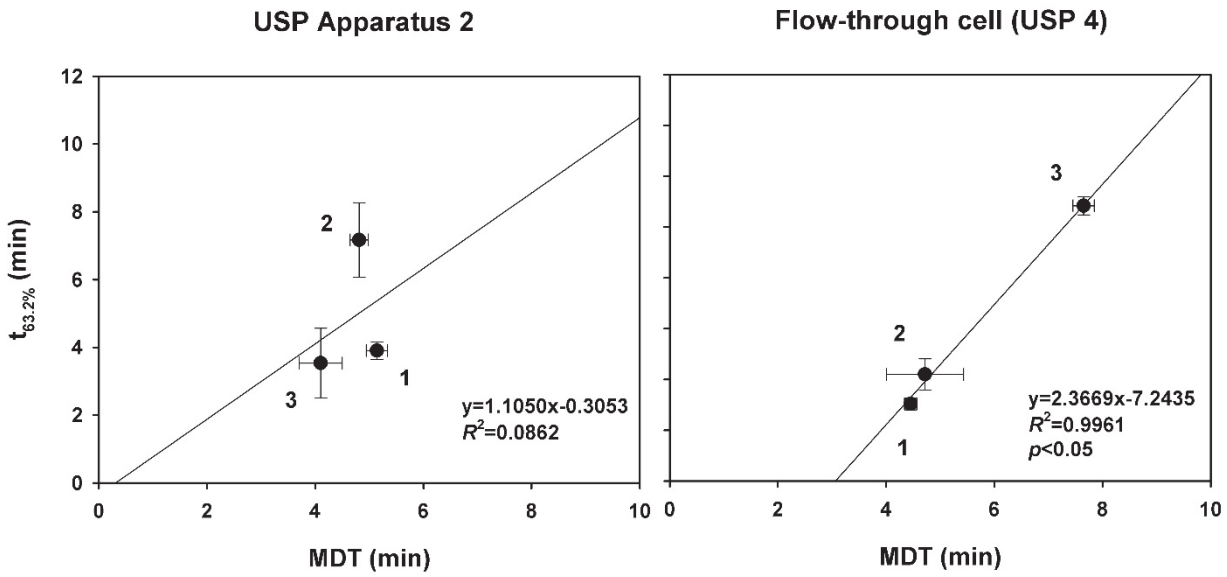

Figure 2. Association between $t_{63.2\%}$ and mean dissolution time (MDT ± SE, n = 6) at pH 1.2 (1), 4.5 (2), and 6.8 (3) in USP apparatus 2 and 4.

2 work under closed finite sink conditions and cannot mimic the hydrodynamic conditions of the GI tract (*11*). Butler and Bateman found that the flow-through cell method showed less variation compared to apparatus 2 and was less dependent on hydrodynamics and the amount of substance tested (*25*).

Apparatus 4 has gained recent acceptance due to its versatility in testing dosage forms where conventional dissolution apparatuses have failed (*26*). The results of this comparative dissolution study for verapamil-HCl tablets agree with those reported by other authors – apparatus 4 was more accurate than USP Apparatus 2. Details of the successful association of MDT and model-dependent parameters of naproxen tablets, ibuprofen suspensions, and fixed-dose combination formulations of acetaminophen and ibuprofen have been reported (*27–29*).

The adjustment to kinetic models was carried out without any physiological meaning to establish a model that describes the dissolution performance of verapamil-HCl tablets under the hydrodynamics of both apparatuses. The aim of adjusting dissolution data is to simplify the analysis and interpretation of drug release as a function of parameters that can be compared by simple statistical tests (*30*).

Prediction of In Vivo Concentration-Time Profile of Verapamil-HCl in Humans

Prediction of in vivo performance of drugs from in vitro dissolution data is essential during drug development. To identify whether the conditions for the flow-through cell reflect the in vivo performance of the drug in humans, it was necessary to predict the in vivo pharmacokinetics and plasma concentration-time profiles of verapamil-HCl from the in vitro dissolution data. A simple convolution method was chosen, utilizing the reported pharmacokinetic parameters of verapamil-HCl (*7, 18, 19*). The predicted plasma concentrations of verapamil-HCl were plotted against the actual published concentrations of Isoptin (Reference) (Fig. 3) (*19*). Pharmacokinetic parameters calculated from the predicted plasma concentrations are listed in Table 3.

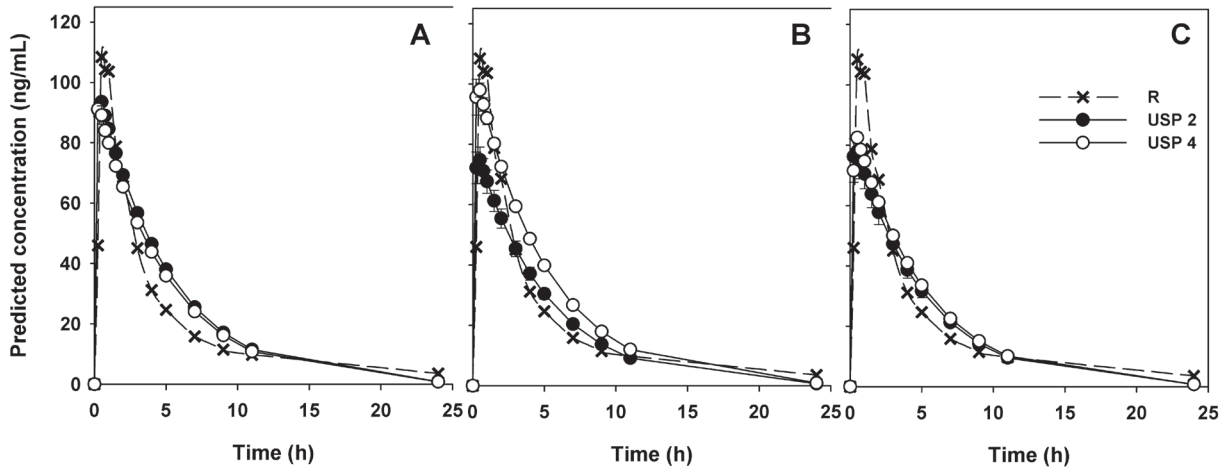

Figure 3. Mean plasma drug concentration-time profiles of verapamil-HCl at pH 1.2 (A), 4.5 (B), and 6.8 (C) (n = 6). R: Reported data from Haeri et al (19).

Table 3. Predicted Pharmacokinetic Parameters of Verapamil-HCl in Humans

		T_{max} (h)	C_{max} (ng/mL)	PE for C_{max} (%)	$AUC_{0\rightarrow\infty}$ (ng·h/mL)	PE for $AUC_{0\rightarrow\infty}$ (%)
Reported data†		0.50	108.4	-	515.8	-
pH 1.2	USP 2	0.50	93.7 (1.0)	13.59	552.2 (5.8)	-7.03
	USP 4	0.25	92.0 (2.5)	15.0	522.9 (6.9)	-1.35
pH 4.5	USP 2	0.50	74.8 (4.2)	31.03	441.6 (25.5)	14.39
	USP 4	0.38	98.7 (2.8)	8.92	574.7 (17.1)	-11.41
pH 6.8	USP 2	0.50	78.6 (5.4)	27.48	460.2 (31.8)	10.79
	USP 4	0.50	82.51 (1.8)	23.93	485.1 (10.4)	5.96

Values are mean (%RSD), n = 6. HCl: Hydrochloric acid; PE: prediction error. AUC: area under the curve.
†: Reported data by Haeri et al. (19).

The predicted curves of the flow-through cell at pH 1.2 and 4.5 were similar to the reported in vivo profile (Table 3). The %PE between the pharmacokinetic data and those calculated by the convolution method, using apparatus 2 at pH 1.2 and apparatus 4 at pH 1.2 and pH 4.5, did not exceed 15% (Table 3). With the flow-through cell, the %PE values between the actual and predicted values for C_{max} and $AUC_{0-\infty}$ at pH 1.2 were 15.0% and –1.35%, respectively, and 8.92% and –11.41%, respectively, for the same pharmacokinetic parameters at pH 4.5. This indicates the validity of the convolution method (*21*). Overall, the flow-through cell was more appropriate for predicting the in vivo performance of verapamil-HCl tablets in humans than USP apparatus 2.

CONCLUSIONS

It is important to study the effect of hydrodynamics from conventional dissolution apparatus together with different media to document the mechanism by which the pharmaceutical dosage form releases particular drugs. The flow-through cell has been tested herein, and it generated satisfactory results for the evaluation of verapamil-HCl tablets, and prediction of in vivo performance was best with data obtained from using the flow-through cell. It is necessary, however, to carry out human bioavailability studies and relate the data to validate these results.

FUNDING

The authors declared no financial support related to this article.

CONFLICT OF INTERESTS

The authors declared no conflict of interest related to this article.

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dx.doi.org/10.14227/DT300423P238

Preparation and Characterization of a Novel Optimum Modified Liquisolid Compact to Enhance the Dissolution Profile of Mifepristone

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ABSTRACT

Most drugs used to treat vaginal disorders are administered orally or parenterally. Mifepristone (MFP) is a Biopharmaceutical Classification System class IV drug that is currently used to abort pregnancies under 70 days long. To improve bioavailability, a modified liquisolid compact (MLSC) formulation has been proposed for vaginal administration for off-label treatment of uterine fibroids. The MLSC was prepared using ultrasonication with pre-screened excipients to minimize the bulk of the final formulation and enhance properties for commercial viability. The MLSC formulation was evaluated for physical properties (including morphology, uniformity, wettability) and in vitro dissolution of MFP. The results showed 85–90% of MFP was released in 90 mins at a pH range of 1.2–7.4, and dissolution in water supports pH-independent dissolution process. Faster dissolution at vaginal fluid pH may minimize the associated adverse effects of the dose. The physical modification of MFP as an MLSC formulation improved dissolution and absorption for potential vaginal administration.

KEYWORDS: Dissolution, powder characterization, site specificity, probe sonication, pH-independent release

INTRODUCTION

Approximately 20–80% of women develop uterine fibroids (UF) by age 50 (*1*). General treatments for UF include invasive therapy and some oral medications like contraceptive pills and progestational agents, or parenteral injections of gonadotropin-releasing hormone (GnRH) agonists, i.e., leuprolide acetate and intrauterine devices. The United States FDA has approved MYFEMBREE (relugolix, estradiol, and norethindrone acetate; Pfizer and Myovant Sciences), a once-daily pill for managing heavy menstrual bleeding associated with UF in premenopausal women, with a treatment duration of up to 24 months (*2*, *3*). Recent domestic and international clinical studies have demonstrated that 3 months of mifepristone (MFP) treatment can significantly reduce the size of UF to achieve complete amenorrhea, improve anaemia-related bleeding, lessen clinical symptoms, and reduce the size of UF (*4*, *5*). Due to the hepatic first-pass effect and low drug solubility at physiological pH, the drug's oral bioavailability is reported to be 40% (*6*).

Conventional oral MFP formulations fail to meet the need for therapeutic concentration and the patient may get dose-related adverse effects (*7*–*9*). Researchers have attempted to increase the physiological availability of MFP by developing various delivery systems, altering the route of administration, and creating multiple carrier systems (*10*, *11*). UF is a localized disorder in which intrauterine distribution through the vaginal site is considered an ideal approach. Although site-specific drug delivery for the localized treatment of UF has not gained much attention, intravaginal MFP administration can improve the treatment of localized disorders (*10*). MFP is considered a Biopharmaceuticals Classification System (BCS) class IV drug on the basis of insufficient permeability of MFP on Caco-2 cells (*11*, *12*). Therefore, augmentation of both solubility and permeability of MFP is essential to get the

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in vivo therapeutic response. The current study aims to enhance MFP's bioavailability with physical alterations and changing the route of administration.

Various methods have been studied to improve drug solubility and dissolution in pharmaceutical formulations, including a liquid-solid compact (LSC) formulation (*9*, *13*–*15*). The amount of bulk in a traditional LSC may be unattainable for designing formulations like tablets, capsules, topical preparation, and others (*16*). Therefore, the modified liquisolid compact (MLSC) formulation was proposed to improve solubility of MFP at the physiological pH range of 1–7.4, using an ultrasonicator and polymer precipitation inhibitor (*15*). The ultrasonicator technique has resulted in maximum solubility augmentation compared to the traditional LSC method (*15*). The MLSC formulation was selected to provide pH-independent dissolution and maximize drug solubility into the compact with a co-solubilizer, potentially resulting in additional bulk reduction and extending commercial viability (*17*).

METHODS

Materials

The MLSC was prepared with blend of polyethylene glycol (PEG) and vitamin ETPGS (d-α-tocopheryl polyethylene glycol 1000 succinate) in a 1:1 ratio (% w/w), then precipitation enhancer (polyvinyl pyrrolidone [PVP] K30, 2% w/v) was added, followed by carrier (Avicel pH 101) and adsorbent (Aeroperl 300) in a 5:1 ratio.

MFP was procured from Pellucid Pharma, Ahmedabad, India. Methanol, propylene glycol (PG), tween 80, PEG 400, PEG 600, glycerin, and PVP K30 were purchased from Loba Chemie (Mumbai, India). Capmul MCM30 was gifted from Abitec (USA). Capryol 90, Lauroglycol, Plurol Oleique, and Avicel (pH 101, 102, and 112) were procured from FMC Biopolymer (Ireland). Aerosil 200, 300, and Aeroperl 300 were obtained from Evonik Industries AG (Germany). Vitamin ETPGS was procured from Sigma Aldrich USA. All the other solvents and reagents used were analytical grade.

Modified Liquisolid Compact (MLSC) Preparation

The process for MSLC preparation is depicted in Figure 1.

Selection of Non-Volatile Liquid Solubilizer

All excipients and solvents were selected, considering their safety, using the inactive ingredient database. The solubilizer was selected using the saturated solubility studies. The solubility of MFP was studied in various non-volatile liquids, including PEG 200, PEG 400, PEG 600, glycerin, tween 80, Capmul MCM30, Lauroglycol, and Plurol Oleique (*18*). Vitamin ETPGS in the concentration range of 0.5–2% w/v and PVP K30 in the range of 1–3% w/v was also studied for MFP solubility in the presence of a selected blend of solvents that act as a precipitation inhibitor (*19*). An ultrasonic processor (VCX 500, Vibra-Cell) was utilized to get maximum solubility into the selected blend; sonication time 10 sec intervals for 5 min at 40 °C.

Selection of Solid Carrier

The binding capacity method was used to select a suitable solid carrier for the liquid blend. Avicel pH 101, 102, and 112 were selected as carrier materials. The addition of 0.1 mL of the liquid blend to 1 g of carrier material was continued until an acceptable range of Carr's index was attained (*18*).

Selection of Adsorbent Material

Flowability of the compact is a valuable attribute for processing into a solid dosage form. Aerosil 200, Aerosil

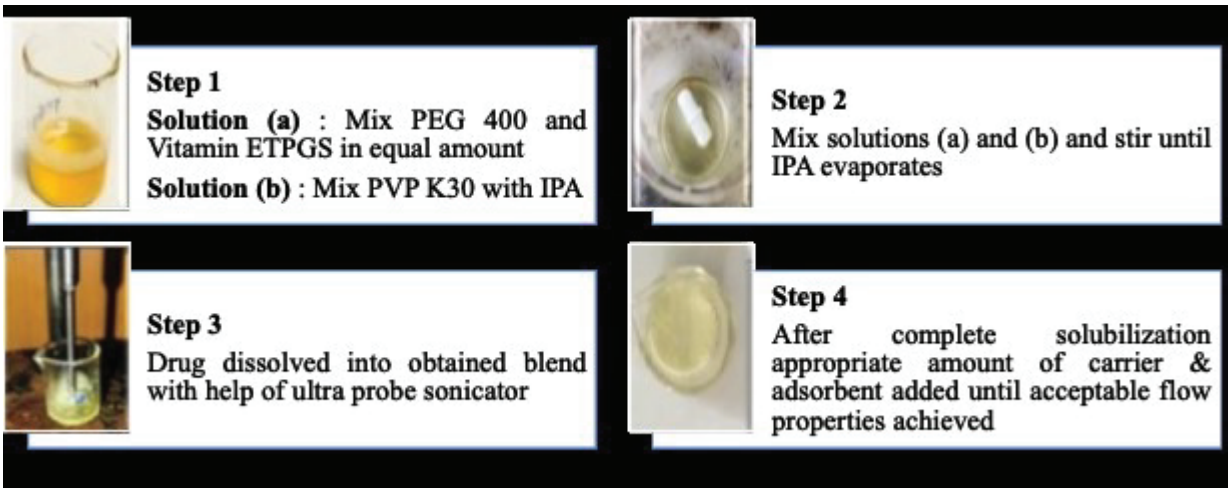

Figure 1. Method for preparation of modified liquisolid compact. IPA: isopropyl alcohol; PVP K30: polyvinylpyrrolidone; PEG: polyethylene glycol.

300, and Aeroperl 300 were screened, and final compacts were evaluated for flow characteristics like Carr's index and angle of repose.

Physical Appearance, Flow Properties, and Drug Content of MLSC

Morphology of the MLSC was assessed using a scanning electron microscope (SEM) (JSM 6010 LA, USA). The samples were mounted on an aluminum stub with double-sided adhesive tape to ensure the specific adhesion of the inserts. A platinum coating was used to reduce thermal disturbance. An applied voltage was used to image the coated samples (*20*). Powder x-ray diffraction (XRD, D2-phase, Bruker) was used to determine the physical state of MLSC. The patterns were documented via Ni-filtered Cu Kα at 40 kV voltage, 20 mA, and steps of 0.02° for 2 seconds, with a scanning speed of 0.01° per second in the intermission 2θ at 10–45 (*21*).

Flow properties (i.e., angle of repose) were determined by the fixed funnel method (*22*).

To assess content uniformity, a 44-mg MLSC, equivalent to 10 mg of MFP, was weighed and solubilized in 10 mL methanol. The solution was placed in a vortex mixer (CM-101 plus, REMI) at 1000 rpm for 2 hours. The solution was filtered by a 0.4-µm syringe filter and measured using a validated high-performance liquid chromatography (HPLC) (W2998 PDA, Waters Alliance, Australia) method with a C8 column reverse-phase (Zorbax SB-C8, 5 µm, 150 × 4.6 mm). The mobile phase was methanol, acetonitrile, water (50:25:25% v/v/v) with a flow rate of 0.8 mL/min, run time 10 min, injection volume 20 µL, and sample 304 nm (unpublished literature) using a linear equation.

Triplicate batches were prepared, and the average value was considered for further characterization.

Fourier Transform Infrared Spectroscopy (FTIR)

Interactions between the drug and excipients in the MLSC were studied using Fourier transform infrared spectroscopy (FTIR) (NICOLET 6700, Thermo Scientific, USA) (*23*). In the analysis, a sample was triturated with KBr before being compacted into pellets (4–5 tons) by the press for 4-5 minutes. The prepared pellet was 10–15% of the formulation with dry KBr. The sample was scanned in the FTIR spectra of 4000-500 cm^{-1}.

Wettability

The wettability of a drug particle significantly affects the dissolution rate of the formulation because wetting is a prerequisite to dissolution (*24*). Wettability of powders was measured using the Washburn method with a tension force tensiometer (Sigma 700/701). The contact angle was calculated from the weight increase over time when the powder sample was in contact with the liquid. Wetting was measured by the change in mass over time during the liquid phase. When the mass starts to remain constant, no more liquid can penetrate, which was considered the endpoint of measurement.

Headspace Gas Chromatography

Residual solvent, i.e., isopropyl alcohol utilized to dissolve PVP K30, is rigorously monitored and regulated at a level that cannot impact drug safety potential. Headspace gas chromatography (Turbo matrix 40 Perkin Elmer) was used to identify the residual solvent in MLSC (*25*). Chromatographic conditions were as follows: Elite 624 column (1.80 µm, 30 m × 0.32 mm), 7 °C/min, injection temp: 210 °C, oven temp: 60 °C, 2-min hold, detector temp: 250 °C, carrier gas: nitrogen, carrier flow: 14 psi. Headspace conditions were as follows: gas temp 80 °C, needle temp 85 °C). A sample was put in a locked vessel and heated to an identified temperature profile. The vapor in the container was tested for analysis.

In Vitro Drug Release

MFP has pH-dependent solubility, so the U.S. Food and Drug Administration recommends two dissolution conditions using apparatus 2 (paddle): 75 rpm with 900 mL of 0.01 N HCl and 50 rpm with 900 mL of pH 1.8 KCl. Maximum solubility is reported at pH 1–3 (*26*). An optimum MLSC formulation was proposed to improve drug release in a physiological pH range of 1–7.4. Dissolution tests were performed with acetate buffer pH 1.2, phosphate buffer pH 4.5, 6.8, and 7.4, and water for vaginal application according to Dobaria et al. (i.e., 25 mL, 50 rpm, 37 ± 0.5 °C, with sampling at 15, 30, 45, 60, and 90 min; 1-mL samples were withdrawn and filtered through a 0.45-µm filter for analysis). For discriminating the dissolution profiles of MFP and MLSC, 0.5% Tween 80 was added to the media. The sample amount withdrawn was replaced with fresh dissolution medium (same volume, kept at 37 °C). Each dissolution test was carried out in triplicate. A validated HPLC method of MFP was performed using an HPLC system (E2695, Waters Alliance) and Empower 3 software, equipped with a photodiode array (PDA) detector, Phenomenex C18 column (5 µm, 250 × 4.6 mm) at 304 nm. The same dissolution method was used for all media.

Dissolution Efficiency

Dissolution efficiency (DE) was calculated as the area under the dissolution curve up to a specific time t, expressed as a percentage of area of the release assay curve and the rectangle that represents 100% dissolution (*28*).

Drug Release Kinetics

The release data from the optimized MLSC formulation were subjected to several kinetic models, i.e., zero-order, Korsmeyer–Peppas, Higuchi, and first-order, to demonstrate the release mechanism of MFP from the MLSC (*21*, *26*).

Stability

Stability tests were performed to determine the formulation's stability and shelf life. MLSC was filled into capsules, packed under aluminum foil, and sealed to represent specific packaging. The optimized MLSC formulation was subjected to an accelerated stability study for 6 months as per ICH Q1(R2) (*29*). Parameters including the angle of repose, physical appearance, drug content, and dissolution were studied during certain intervals.

RESULTS AND DISCUSSION

MFP's maximum solubility (15–16 mg/mL) was achieved in PEG 400. With the help of ultrasonication, drug particle size was reduced, and solubility increased to 90–100 mg/mL. Co-solubilizers (vitamin ETPGS and PVP K30) were added during ultrasonication to reduce bulk and improve solubility. Solubility study results with PEG 400, vitamin ETPGS, and PVP K30 showed maximum solubility augmentation (290–300 mg/mL) compared to PEG 400 alone. A blend of PEG 400, vitamin ETPGS, and PVP K30 in the ratio of 1:1:2 was used for the optimized MLSC formulation.

Binding capacity was used to select the suitable carrier material for the liquid blend of the drug. Avicel pH 101 was selected as it showed the highest binding capacity (0.5 mL/g), whereas Avicel pH 102 and pH 112 had 0.2 and 0.17 mL/g binding capacity, respectively. Aeroperl 300, having the lowest Carr's index (13.24), was selected as an adsorbent material to impart flow properties, whereas Aerosil 200 and 300 had 15.02 and 17.74 Carr's index values, respectively.

Physical Properties of MLSC

SEM and XRD studies showed reduced crystallinity of MFP in the MLSC formulation compared to the pure drug, as shown in Figure 2.

The SEM image of MLSC shows reduced crystallinity of MLSC compared to the drug. On the other hand, the XRD pattern of the drug revealed prominent peaks at 21.2°, 16.4°, 18.6°, 20°, 21.5°, 23.2°, 26.5°, demonstrating MFP's crystalline nature. The XRD pattern of the MLSC exhibited weak peaks compared with the pure drug. Partial amorphization of the drug was seen in the range of 20–30°, which was due to presence of the hydrophilic chain of vitamin ETPGS.

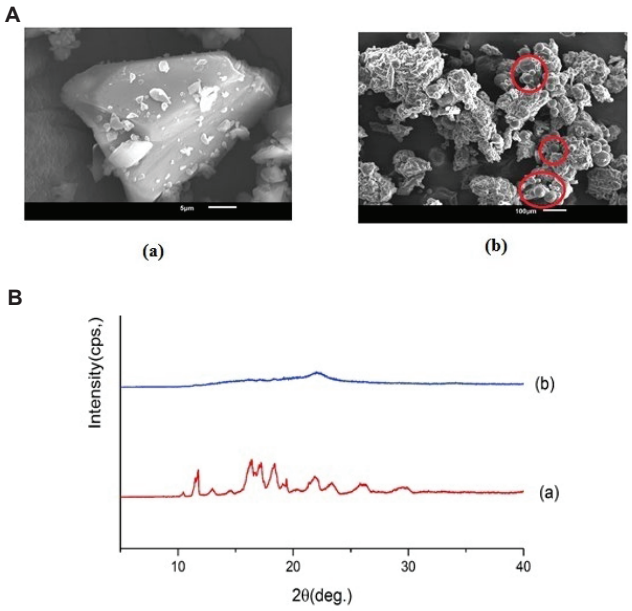

Figure 2. (A) Scanning electron microscope (SEM) images and (B) x-ray diffraction (XRD) graph of (a) mifepristone and (b) modified liquisolid compact formulation.

Flowability was accessed using the angle of repose. Values in the range of 25–27 suggest excellent flow properties. Drug content was 98–99%, which is within the appropriate specification range for uniformity per *Indian Pharmacopoeia*. The yield from triplicate batches was 95–97%, indicating the suitability of the preparation method.

FTIR

All characteristic peaks of MFP were retained in the final MLSC formulation, which shows the drug's compatibility with the formulation.

The FTIR spectra of the drug showed distinctive peaks, which comprised peaks at 3381 cm^{-1} for -OH (hydroxyl group), 2878 cm^{-1} for C-H stretching (methyl and methylene groups), and at 1655 and 1517 cm^{-1} for C-H stretching (aromatic nucleus). In the optimized batch FTIR, the analysis revealed lower intensity of existing characteristic peaks of MFP, which confirmed the absence of any physical interaction with excipients used in the preparation of MLSC.

Wettability

Using the Washburn method at the end of 5 minutes, the maximum weight gain observed for the drug was 300 mg, whereas for MLSC it was 530 mg. This shows increased wettability of the MLSC formulation compared with pure MFP. This observation may be related to augmentation

of the drug's solubilizer blend, which could further act as a surface-active agent and reduce hydrophobicity of the drug particles.

Headspace Gas Chromatography

In the MLSC composition, isopropyl alcohol was used as an organic solvent to dissolve PVP K30. The limit was 0.83 ppm, which is acceptable for pharmaceutical formulations (i.e., limit of 5000 ppm) (*30*, *31*).

In Vitro Drug Release

Comparative in vitro drug release profiles of the drug and MLSC in various dissolution media are shown in Figure 3. Drug release from API dispersion was approximately 4.5% after 90 min due to the presence of Tween 80. In vitro drug release profiles of the drug at various pH levels show the pH-dependent solubility of the drug. In comparison with MFP, the dissolution rate of all MLSC formulations was remarkably enhanced. Possible reasons for the improvement in dissolution include conversion of MFP from its crystalline to amorphous state and improved wettability (*32*).

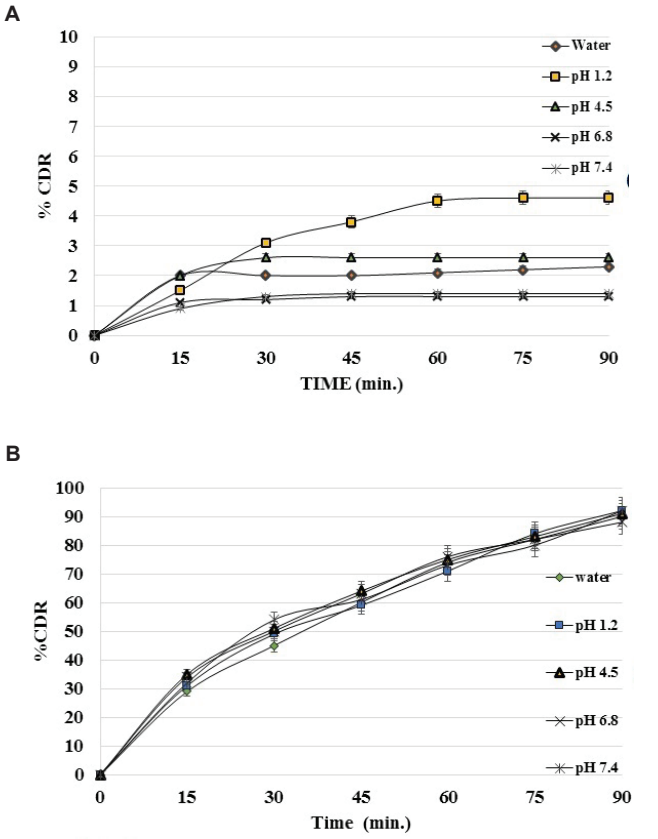

Figure 3. In vitro dissolution profile of the (a) mifepristone and (b) modified liquisolid compact formulation. CDR: cumulative drug release.

Dissolution Efficiency

DE values at various pH levels are shown in Table 1. The DE of MFP and MLSC were in the range of 0.21–0.76% and 14.66–15.33%, respectively. This further indicates excellent improvement in the dissolution rate of MFP from prepared MLSCs as compared to other methods reported for dissolution enhancement of MFP (*33*, *34*).

Table 1. Dissolution Efficiency (DE) of Drug and MLSC at Various pH Levels

Dissolution Medium pH	DE of MFP (%)	DE of MLSC (%)
1.2	0.76	15.33
4.5	0.43	15.16
6.8	0.21	14.66
7.4	0.23	15.33
Water (7.0)	0.38	15.33

MFP: mifepristone; MLSC: modified liquisolid compact

Drug Release Kinetics

The drug release mechanism follows the zero-order model (R^2 = 0.9233), which indicates pH-independent release of MFP from the MLSC.

Stability

After 6 months, none of the parameters deviated from their acceptable range. Dissolution was carried out in water, considering the pH-independent dissolution of MFP from the MLSC formulation. No significant changes were observed in the rate of dissolution at selected sampling intervals, as shown in Table 2. Therefore, the MLSC formulation was stable.

Table 2. Stability Study of the MLSC Formulation

Parameters	0 month	3 months	6 months
Appearance	Yellowish white	Yellowish white	Yellowish white
Angle of repose	25 ± 1	26 ± 1	26 ± 1
Cumulative drug release (%)			
15 min	35 ± 2	36 ± 2	35 ± 2
45 min	64 ± 2	65 ± 2	64 ± 2
90 min	91 ± 2	90 ± 2	90 ± 2
Assay %	99.2 ± 1	99.3 ± 1	99.2 ± 1

** CDR: Cumulative Drug Release; MLSC: Modified liquisolid compact*

CONCLUSION

Popular treatments for UFs include surgery, intramuscular injectable formulations, and various oral medications. The impact of adding MFP to the targeted delivery route may be limited by its solubility. The physical modification of MFP into an MLSC formulation successfully improved dissolution and absorption for potential vaginal administration, which may improve efficacy of treatment for UF and reduce dose-related size effects.

FUNDING

This research received a Scheme of Developing High-Quality Research grant (ref no. 201901300005) from the Knowledge Consortium of Gujarat.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest and financial disclosure concerning this study.

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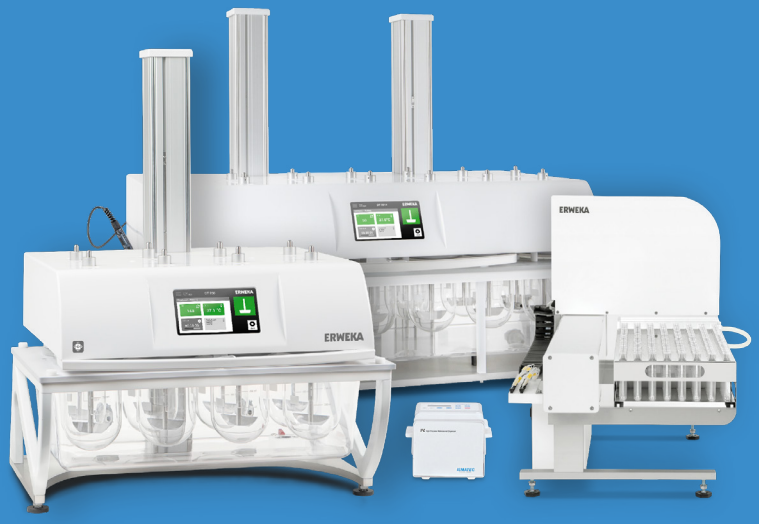

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dx.doi.org/10.14227/DT300423P246

Development of In Vitro Dissolution Test Method for Bilastine and Montelukast Fixed-Dose Combination Tablets

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ABSTRACT

Bilastine and montelukast are Biopharmaceutical Classification System (BCS) class II compounds with low bioavailability, especially when taken orally. It is challenging to develop a dissolution test. The purpose of this study is to select an in vitro dissolution test that would be useful for bilastine and montelukast solid oral dosage forms. Solid oral dosage forms containing bilastine and montelukast, for which in vivo data are available, were studied using different in vitro dissolution test conditions. Dissolution tests were performed under sink conditions in various non-biorelevant media with changes in various parameters like United States Pharmacopeia (USP) basket or paddle apparatus, rotation speed, volume of media, with different dissolution media. A novel high-performance liquid chromatography method was developed and validated to simultaneously estimate the dissolution profile. The optimal dissolution conditions for bilastine and montelukast are 900 mL 0.5% sodium lauryl sulphate (SLS) in water at 37 ± 0.5 °C and 75 rpm using the paddle apparatus.

KEYWORDS: Montelukast, bilastine, dissolution, fixed-dose combination

INTRODUCTION

Bilastine (BLS) is a new second generation H1 antihistamine drug substance that reduces allergic rhinitis and urticaria, functioning as an antiallergenic agent (*1*). Montelukast sodium (MTK) is a potent, selective cysteinyl leukotriene receptor antagonist that inhibits bronchospasm (*2*). Combining BLS and MTK reduces severe acute respiratory syndrome (SARS) symptoms and improves long-term quality of life of patients with asthma (*3*). This combination drug product is currently sold in India in tablet form under the brand names of Billargic M (Synokem Pharmaceuticals), Antegy M (Intas Pharmaceuticals), and Bilamove M (Synokem Pharmaceuticals), containing 20 mg BLS and 10 mg MTK.

Official monographs of the *Indian Pharmacopoeia and British Pharmacopoeia* describe dissolution testing procedures for quality control of MTK drug products (*4*, *5*). No official monograph exists for BLS. Various analytical methods have been developed for quality control testing of BLS and MTK (*6–9*). However, an in vitro dissolution method has not been developed for BCS class II drugs with low solubility and high permeability, such as BLS and MTK solid oral dosage forms. The dissolution test studies the drug's gradual release into a dissolution media. It is crucial to evaluate several elements that may have an impact on the dissolution rate. For example, agitation speed affects the diffusion layer's thickness and reflects the gastrointestinal tract's peristaltic motions (*10*, *11*).

This investigation aims to find the optimal dissolution conditions for release of BLS and MTK from solid oral dosage forms and develop a reverse-phase high-performance liquid chromatography (HPLC) method for simultaneous estimation of BLS and MTK content in dissolution samples.

METHODS

Chemicals

BLS and MTK working standards were received as gift

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samples from Synokem Pharmaceuticals, Haridwar, Uttarakhand, India. The marketed formulation of BLS + MTK combined tablets was procured from the local pharmacy. Methanol, acetonitrile, triethylamine, orth phosphoric acid were of HPLC grade from Merck. Sodium lauryl sulphate, ammonium acetate, glacial acetic acid, hydrochloric acid (HCl), potassium dihydrogen phosphate were of analytical grade.

Preparation of Standard Solution

Stock standard solutions of MTK and BLS (190 µg/mL and 380 µg/mL, respectively) were prepared by dissolving the appropriate amount of working standard in diluent. Working solutions of standard of MTK and BLS (11 µg/mL and 22 µg/mL, respectively) were prepared by adequately diluting the stock solution with respective dissolution media.

Analytical Method Development

An HPLC system (1260 Infinity II, Agilent) with a photo diode array detector was used for analysis. The initial method development was done by trial and error, injecting blank and standard solutions for peak detection and different trials with varying mobile phase buffer ratio, flow rate, and gradient. The reversed phase chromatographic conditions included a Zorbax eclipse plus C18 column (150 mm × 4.6 mm, 5 µm) as a stationary phase, 0.05 M ammonium acetate buffer (pH 5.2) using glacial acetic acid as mobile phase A, and a mixture of methanol and water (90:10 %v/v) as mobile phase B, at 1.2 mL/min in gradient mode of separation. The injection volume was 50 µL, and chromatograms were recorded at 280 nm using a column oven temperature of 25 °C. A homogenous mixture of methanol and ammonium acetate buffer (pH 5.2) (75:25 %v/v) was used as a diluent. The gradient conditions are given in Table 1.

Table 1. HPLC Gradient Elution Conditions

Time (mins)	Mobile Phase A (%)	Mobile Phase B (%)
0.0	40.0	60.0
1.0	40.0	60.0
3.0	10.0	90.0
9.0	10.0	90.0
12.0	40.0	60.0
15.0	40.0	60.0

HPLC: High-performance liquid chromatography.

Analytical Method Validation Protocol

The analytical method was validated for specificity, repeatability, precision, linearity, recovery, and stability in aqueous solution according to International Council for Harmonization (ICH) guidelines (*12*).

Repeatability was determined by analyzing six replicates of same solution containing 11.1 µg/mL of MTK and 22.2 µg/mL of BLS at the 100% level.

Precision was evaluated by repeating the dissolution test with six replicates (method precision). In addition, intermediate precision was evaluated by repeating the dissolution test with six replicates on a different day by a different analyst with a different column on another HPLC system.

Linearity was evaluated using five different concentrations ranging from 5.5–16.6 µg/mL for MTK and 11.1–33.3 µg/mL for BLS, corresponding to 50%–150% of sample concentration.

Recovery was evaluated in triplicate at three different levels (50%, 100%, and 150%) of sample concentration using standard addition method.

Stability of MTK and BLS was evaluated using the standard solution over a 48-hour period while stored at room temperature (at 37 ± 0.5 °C). Sample solutions were prepared using the same dissolution media and conditions as those used as the dissolution test. The drug concentrations in samples at 0, 12, 24, and 48 hrs were measured and compared.

Dissolution Method Development

Preliminary tests were ran to select the dissolution media. Solubility of BLS and MTK were determined in 0.1 N HCl, purified water, and pH 6.8 phosphate buffer. Purified water, 0.1 N HCl, and 0.05 M phosphate buffer (pH 6.8) were selected for development trials, and varying concentrations of sodium lauryl sulphate (SLS) were incorporated (0.2%, 0.5%, and 1.0%) as a surfactant. Media volumes of 500 and 900 mL were evaluated for feasibility.

Six BLS and MTK fixed-dose combination tablets were weighed and transferred into individual bowls containing dissolution media maintained at 37 ± 0.5 °C. The dissolution tests were carried out using an Electrolab dissolution apparatus (EDT 08Lx) with auto sampling mechanism, fitted with the United States Pharmacopeia (USP) basket or paddle apparatus (apparatus 1 or 2, respectively), at 75 or 100 rpm. The dissolution test was performed using different dissolution media. Samples (10 mL) were collected at 10, 20, 30, 45, and 60 mins from the midway zone between the wall of the vessel and top of paddle, not less than 1 cm from the vessel wall. Each sample was filtered through a 0.45-µm polyvinylidene difluoride (PVDF) syringe filter. The first 5 mL of filtrate

was discarded to saturate the syringe filter, and the other 5 mL of filtrate was collected and analyzed.

RESULT AND DISCUSSION

HPLC Method Validation

Results of the HPLC method validation parameters are presented in Table 2.

Table 2. Method Validation Results

Parameters	Specifications	Montelukast	Bilastine
System suitability	NMT 2.0 %RSD	1.15	0.92
Method precision	NMT 2.0 %RSD	0.64	0.76
Intermediate precision	NMT 2.0 %RSD	1.60	1.77
Linearity	$R^2 > 0.99$	$R^2 = 0.9997$ (49.05x + 1.725)	$R^2 = 0.9992$ (40.652x + 275.09)
Recovery levels			
50%	95–105%	99.5%	99.1%
100%	95–105%	99.9%	100.2%
150%	95–105%	99.5%	99.4%

NMT: not more than; RSD: relative standard deviation.

System Suitability

A system suitability parameter was established by injecting five replicate injections of standard solution. The %RSD values for MTK and BLS were 1.15 and 0.92, respectively. The chromatographic parameters were within the ICH stated range, having retention times of 3.7 and 6.7 mins for MTK and BLS, respectively.

Specificity

The method was specific, with no interference of excipients and blank (dissolution media) at the retention time of analyte peaks.

Precision

The %RSD values for precision must be less than 2.0%, and the absolute difference between method precision and intermediate precision values should not exceed 3.0%. All values were within the acceptable range. The %RSD values for repeatability with MTK and BLS were 0.64 and 0.76, respectively.

Linearity

A linear relationship was obtained between mean peak area under the curve (AUC) and concentration of the drug in the range of 5.55–16.65 µg/mL for MTK and 11.1–33.3 µg/mL for BLS. The calibration curve of MTK and BLS was obtained by plotting the graph between mean peak AUC against concentration (µg/mL). The correlation coefficient (R^2) for MTK and BLS were 0.9997 and 0.9992, respectively.

Recovery

Recovery was evaluated in the range of 50%, 100%, and 150% of drug concentration MTK (5.56–16.67 µg/mL) and BLS (11.10–33.33 µg/mL). The recovery values were within the expected range of 95–105%.

Solution Stability

During this study, only a 0.8% and 0.6% change in the concentration of MTK and BLS was observed from the initial value following up to 24 hours of storage at room temperature (25 °C).

Filter Compatibility Study

A filter compatibility study was conducted to compare the percentage of drug release in sample solutions filtered through different syringe filters with that of the control solution, which was centrifuged. Based on the %RSD criteria for both BLS and MTK sample solutions, the 0.45-µm PVDF syringe filter, 0.22-µm PVDF syringe filter, and 0.45-µm nylon filter (SY25NN) were deemed suitable, as they exhibited a percentage deviation of drug release below 1.5% compared to the control solution. Consequently, the 0.45-µm PVDF syringe filter was used to filter sample solution throughout study.

Optimizing the Dissolution Method

Figure 1 shows the dissolution profiles of MTK and BLS in three different dissolution media. Water as a dissolution medium had the fastest drug release rate compared to others. Because BLS+MTK is a class II drug, the incorporation of surfactant plays a crucial role in the solubility of drugs during the dissolution test. Various concentrations of SLS in water were studied to optimize the concentration of SLS in the dissolution medium. The drug release profile at 60 minutes showed that 0.5% SLS in water is the most suitable medium for dissolution.

Figure 2 shows the dissolution profiles of MTK and BLS in apparatus 1 or 2 with different agitation speeds (75 and 100 rpm) and media volumes (500 and 900 mL). Using apparatus 1 (basket) at 75 and 100 rpm) did not generate enough force for complete drug release from the tablet formulation after 60 min of dissolution. Apparatus 2 (paddle) was then used (also at 75 and 100 rpm) to maximize the rate of drug release along with discrimination power. A satisfactory outcome was achieved with apparatus 2 at 75 rpm, with a gradual increase in drug release over 60 min. Media volumes of 500 and 900 mL were tested to evaluate feasibility of the drug's release profile as a class 2 drug. The use of 900 mL was favorable to achieve the criteria of sink condition and better drug solubility.

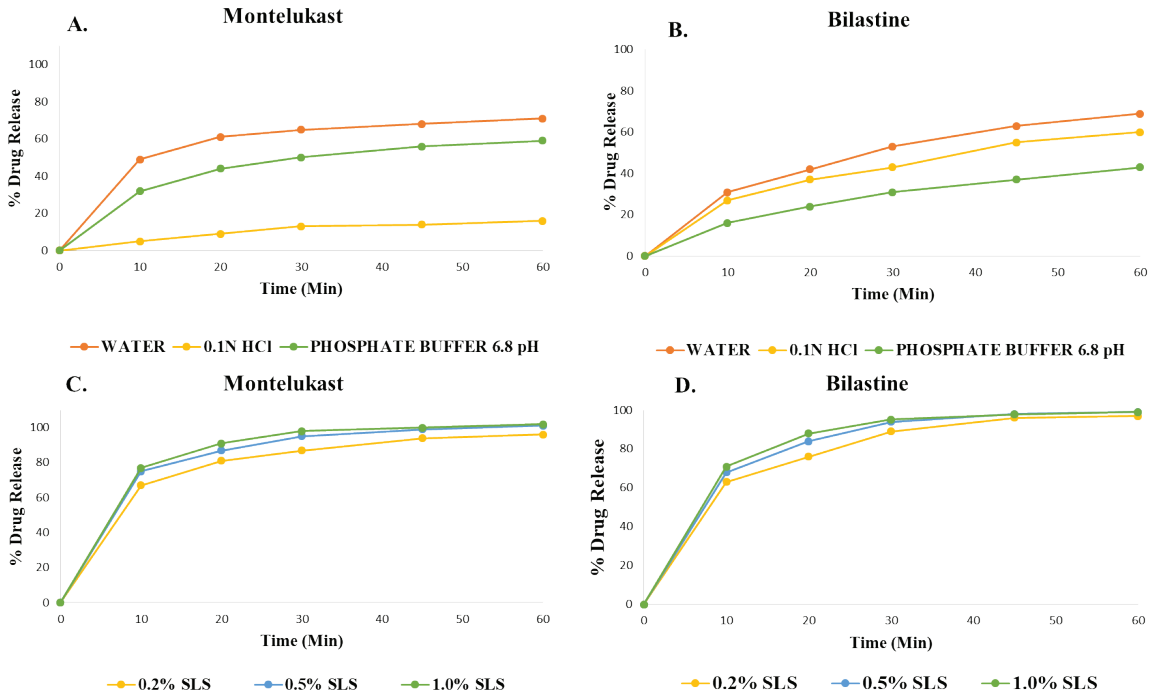

Figure 1. Dissolution profiles of montelukast and bilastine in different media (A and B) and sodium lauryl sulphate (SLS) concentrations (C and D).

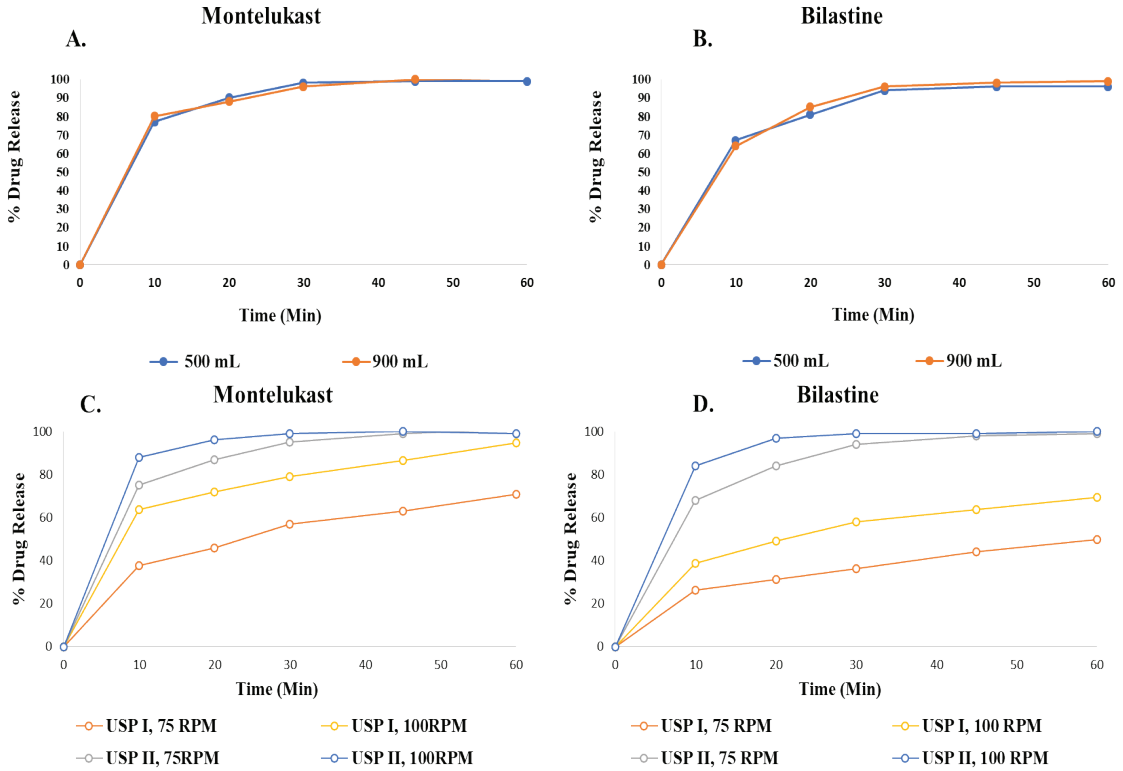

Figure 2. Dissolution profiles of montelukast and bilastine in different media volumes (A and B) and apparatus/stirring speeds (C and D). USP: United States Pharmacopeia.

The optimal dissolution conditions for BLS and MTK are 900 mL 0.5% SLS in water at 37 ± 0.5 °C using the paddle apparatus at 75 rpm.

CONCLUSION

The objective of this study was to develop and validate dissolution method for MTK and BLS fixed-dose combination tablets. Several factors were investigated to determine the optimal method. The most robust dissolution conditions were recorded using apparatus 2 (paddle) with 900 mL of 0.5% SLS surfactant in water as dissolution medium at 37 ± 0.5 °C and 75 rpm. MTK and BLS were found to be stable for 24 hrs, indicating good stability of the drug in dissolution medium. The relatively shorter run time (15 min) for both drugs facilitates rapid estimation of drug release in dissolution samples during routine analysis. The optimized dissolution test conditions proved to be adequate, reliable, and feasible, and all parameters evaluated in this study met the USP acceptance criteria. This method could be considered for future official pharmacopeial methods and for studies in the pharmaceutical industry where MTK and BLS dissolution is required.

ACKNOWLEDGEMENTS

We would like to sincerely thank Graduate School of Pharmacy, Gujarat Technological University for providing required laboratory support and infrastructure to carry out this work smoothly.

FUNDING

The authors disclosed no financial support related to this article.

CONFLICT OF INTEREST

The authors disclosed no conflicts of interest related to this article.

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dx.doi.org/10.14227/DT300423P252

Virtual Workshop Report: Approaches, Regulatory Challenges, and Advances in Bioequivalence, Dissolution Testing, and Biowaivers: Manila, Philippines, February 22–24, 2023

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INTRODUCTION

The virtual workshop, "Approaches, Regulatory Challenges, and Advances in Bioequivalence, Dissolution Testing, and Biowaiver," was held February 22–24, 2023, via the Zoom online platform. The conference was co-organized by the University of the Philippines College of Pharmacy (UPCP) and the American Association of Pharmaceutical Sciences-In Vitro Release and Dissolution Testing (AAPS-IVRDT) Community. The webinars were chaired by Vivian Gray (AAPS) and Dr. Bienvenido S. Balotro (UPCP) with Drs. Jie Shen, Nikoletta Fotaki, Imelda G. Pena, and Leonel Santos, and Assistant Profs. Jean Flor Casauay, Ethel Ladignon, Clinton Gomez, and Czarina Dominique R. De los Santos as members of the organizing committee.

The 3-day webinar series consisted of three scientific sessions on basic principles, challenges, and advances in dissolution technologies, bioequivalence (BE), and biowaivers.

Each session was followed by an open forum of the speakers and the participants. Pharmacists who completed the 3-day virtual workshop earned Continuing Pharmacist Education units.

The objectives of the webinars were as follows:

- Learn best practices in developing discriminating methods and increase knowledge of drug product characterization and dissolution testing
- Explore new concepts of modeling to support dissolution specifications
- Develop networking for research collaboration, knowledge sharing, education, and industry exchange in dissolution, biowaiver, and BE topics.

On the first day of the webinar series, there were 909 participants. On the second day, 778 attendees joined the event, and on the third and last day of the webinars, there were 754 participants. The participants included members of industry, regulatory and government agencies, professional organizations in pharmacy practice, academia, and other professionals interested in dissolution, BE, and biowaivers.

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Day 1: Basic Principles

Day 1 began with welcome remarks and a program overview given by Vivian Gray and Dr. Bienvenido S. Balotro, respectively. Both served as co-chairs of the organizing committee. The first day of the program was moderated by Dr. Imelda G. Pena, who also presided over the open forum after the presentations. The first talk was by Dr. James E. Polli from the University of Maryland who presented "Biopharmaceutics Classification System (BCS)-Based Biowaivers ICH M9."

Dr. Polli's presentation introduced chemistry, manufacturing, and controls (CMC) activities and discussed some elements of the International Council for Harmonization (ICH) BCS M9 guidance. According to Dr. Polli, the ICH BCS M9 guidance was finalized in 2020 and is recognized worldwide. In M9, immediate-release (IR) oral dosage formulations of BCS Class I and III drug products with the same strength as the reference product may be eligible for a biowaiver. For about half of all drugs, in vitro testing to assess BE is globally acceptable. In vitro studies are sometimes better than conventional in vivo pharmacokinetic studies for assessing BE of IR solid oral dosage formulations. M9 is a notable step forward, as it is the first harmonized allowance of BCS-based regulatory relief, including in Japan.

Dr. Polli cited the importance of CMC activities during drug development and product life cycle management for product understanding, quality, and manufacturing. Although typically invisible to prescribers and patients, CMC activities allow ongoing product manufacturing and product quality control while implementing product lifecycle changes, such as excipients, process, and/or manufacturing location.

Dr. Polli also discussed ICH BCS M9 guidance on solubility, permeability, and excipients. Regarding solubility, M9 requires that the highest dose is soluble in 250 mL of aqueous media over the pH range of 1.2–6.8 at 37 ± 1 °C, where the highest dose is not necessarily the highest formulation strength (e.g., a tablet with 250 mg of drug substance) but the highest single therapeutic dose (e.g., two 250-mg tablets). Given that M9 is an alternative to an in vivo human BE study where presumably a single unit of the highest formulation strength is tested, the basis for preferring the highest single therapeutic dose dissolves in 250 mL to be highly soluble is not well described. However, M9 indicates that if the highest dose does not meet this criterion, but the highest strength does, additional data should be submitted to justify the BCS-based biowaiver approach.

With regards to permeability, M9 allows reliance on the Caco-2 monolayer method. Dr. Polli cited a recent workshop report that discussed the importance of global acceptance of permeability methods, opportunities to expand the use of biowaivers (non-Caco-2 cell lines, totality-of-evidence approach to demonstrate high permeability), and the future of permeability testing.

Dr. Polli also discussed the differences of excipients used between test and reference products in M9 related to drug class.

Dr. Zhao Liu (Merck) was the second speaker who presented on method development and setting clinically relevant dissolution specifications including the Quality-by-Design (QbD) approach. Dissolution testing serves as an important tool to guide formulation design and product assessment (and is required for quality control) and surrogate for bioperformance if an in vivo correlation is established. Commonly used compendial dissolution equipment, i.e., United States Pharmacopoeia (USP) apparatus 1–7, which have been harmonized among USP, European Pharmacopoeia (EP), and Japanese Pharmacopoeia (JP), are used for different dosage forms based on their properties and intended use. Different detection methods (spectrometric and chromatographic) were compared, and their advantages and applications were discussed. Spectrometric detection is rapid but needs to demonstrate specificity, whereas the chromatographic method requires more time and expensive equipment but has a wider dynamic range. Automated sampling equipment has been increasingly used for dissolution testing, which also needs to be assessed and compared to manual sampling.

Dr. Liu also discussed a commonly observed dissolution issue known as coning, which is caused by an artifact of the dissolution vessel and hydrodynamics of the dissolution media. The current solution, i.e., apex or peak vessel, can efficiently solve the issue.

Finally, Dr. Liu talked about dissolution as a critical aspect of the QbD approach in drug product and method development. This includes consideration of the properties of the active pharmaceutical ingredient (API), such as BCS class, pKa, solubility, dose range, and whether the product is the salt form. For IR oral dosage forms, particle size distribution, solubility, and diffusion coefficient of the API are critical to the dissolution rate, according to the first principle of API dissolution mechanism (Nernst–Brunner and Noyes-Whitney theories). Formulation of critical quality attributes (CQAs) and manufacturing

critical process parameters (CPPs) can affect drug product dissolution performance, including raw materials (API and excipients), blending and lubrication, compression, and film coating, all of which can be assessed using a fishbone diagram. In addition, analytical method parameters are critical, such as the dissolution apparatus, rotational speed, media, and surfactant selection. In general, the strategy of dissolution method development is based on BCS classes, i.e., for class 1 and 3 are highly soluble compounds, FDA guidance should be used. For class 2 and 4, as well as class 1 and 3 drugs that do not meet FDA guidance, dissolution might be the rate-limiting step for absorption. For amorphous solid dispersion formulations, dissolution can be utilized to detect crystalline API content in the drug product.

The third speaker was Vivian Gray (*Dissolution Technologies*), who presented "Challenges when Developing a Discriminatory Dissolution Method and Aspects of Method Validation." Vivian began with defining a "discriminatory" method and why it is necessary, reiterating that discriminating methods can contribute to specifications that distinguish between bioequivalent and bio-inequivalent batches. She reviewed the necessary characteristics of a discriminatory method and gave resource materials with regulatory and industry expectations. The primary references were European Medicines Agency (EMA) reflection papers and *USP* chapter <1092> The Dissolution Procedure: Development and Validation. Vivian outlined how to develop a discriminatory method. The first step is to identify CQAs related to the drug substance, drug formulation, and drug product manufacturing process. She gave examples in each category. The second step is to identify which of these attributes affect the in vivo release. The third step is to manufacture a drug product that reflects the upper and lower limits (± 20%) of that variable, ideally about two or three variations for each category (drug, drug formulation, manufacturing process). The fourth step is to run these variation products, preferably one variable at a time versus the target product. Lastly, compare the dissolution profiles and determine if there are significant differences among the variables and the target. Hopefully, there will be at least two or three variables that the method can pick up differences for. If not, then go to a backup method that is possibly more complex and may not achieve sink conditions. In addition to a discriminatory method, there should be an in vivo linkage element to the in vitro method data.

Vivian went on to discuss validation aspects related to the sample analysis. This includes the critical validation discriminatory method, which should have an in vivo linkage element to the in vitro discriminatory method parameters of linearity, selectivity, robustness, accuracy, intermediate precision, carryover, filter selection, sinkers, and stability. Robustness and intermediate precision are early indicators of issues that could develop in method transfer. The importance of critical factors in the testing method was emphasized.

Vivian ended her presentation by sharing resources available to the dissolution analyst. This includes websites for the USP Pharmacopeial Forum and USP dissolution compendial tools, the AAPS website with access to several journals, FDA dissolution methods database, and USP dissolution methods database. She also provided the website for *Dissolution Technologies* journal, adding that the website is searchable and open access. She also gave a list of books of interest.

The first day of the program ended with an open forum moderated by Dr. Imelda G. Pena.

Asst. Prof. Jean Flor Casauay gave a synopsis of Day 1 and introduced the opening of Day 2.

Day 1 virtual workshop attendees and speakers.

Day 2: Challenges

Willison de Luna from the Philippine Food and Drug Administration (FDA) gave the first talk on the second day on "Regulatory Challenges on Dissolution, BA/BE and Biowaivers: The Philippine Experience." Republic Act 9711 and its Implementing Rules and Regulations mandates the FDA to ensure that all drug products comply with the standards of quality, safety, and efficacy. In line with this, Mr. De Luna said that a satisfactory BE study report or biowaiver shall be provided as proof of product interchangeability with the reference or innovator drug product. This is required prior to issuance of a marketing authorization, i.e., Certificate of Product Registration, for a generic drug product in the Philippines. In addition, the FDA conducts inspections of BE testing centers and

clinical laboratories handling the clinical, bioanalytical, and statistical phases of BE studies.

The FDA faces various challenges in implementing the guidelines on product interchangeability. Currently, the number and technical capacity of evaluators handling product dossier review including equivalence studies and inspectors of BE testing centers needs to be augmented. In addition, the coverage of drug products requiring BE studies is limited as the guidelines in the Philippines currently covers oral solid dosage forms only. On the other hand, industry stakeholders encounter difficulties in the conduct and compliance with BE studies, particularly in the costs and expenses related to the BE studies, such as the conduct of clinical trials, procurement of reference drug products (especially for those not registered in the Philippines), and validation of bioanalytical methods. These factors may affect compliance with the requirements and guidelines for registration, leading to delayed availability of generic drug products in the market.

Dr. Andreas Abend presented the second talk entitled, "Challenges with Dissolution Similarity Assessment." He stated that assessing the impact of manufacturing changes on product quality is an important part of pharmaceutical product lifecycle management. Formulations that were used in clinical trials to establish safety and efficacy, or generic drugs that are deemed equivalent to a reference listed drug, all sooner or later experience changes in their composition and or manufacturing process. Thus, it is critical to have reliable tools to ensure that these changes do not negatively impact product quality. A major concern is that such changes may negatively impact drug in vivo performance, resulting in poor efficacy or safety or both. Rather than performing unnecessary clinical studies, industry and regulators rely on dissolution testing to assess potential negative impacts of certain manufacturing changes. The level of testing and the acceptance criteria required to assess the effect of manufacturing changes on in vivo performance is proportional to the risk to the patient. The US FDA's Scale-up and Post approval Change (SUPAC) guidance documents classify changes as minor, moderate, and major. For minor and moderate changes, in vitro dissolution testing is generally accepted to assess the impact of changes; if the required acceptance criteria are met, then the changes are supported. In contrast, major changes, which are likely to impact bio-performance, typically require demonstration of BE before the changes are approved. Changes that are unlikely to impact bio-performance (i.e., minor changes) are supported when the approved quality control dissolution specifications are met. For moderate changes that could impact bio-performance, comparative dissolution testing is typically required.

The amount of dissolution testing required to support formulation changes for IR products further depends on the physicochemical properties of the API. Drugs with high aqueous solubility belonging to the BCS class 1 and 3 are considered low risk, and if their dissolution rates are not considered very rapid (i.e., 85% dissolved is not released within 15 min), then dissolution profiles generated in a single aqueous medium are usually evaluated for similarity. In case of poorly soluble drugs, further distinctions are made between drugs with high permeability (i.e., more than 85% absorbed after oral administration) and low permeability (those not meeting this criteria). Even moderate formulation changes require BE studies for drugs belonging to BCS class 4. However, for class 2 drugs, dissolution profile comparisons in four aqueous media plus water are required (use of surfactants is not allowed). A differentiation based on the BCS class does not apply for manufacturing changes such as site, process, or scale. Nevertheless, these changes are also categorized as minor, moderate, and major, and the level of data to support these increases with increasing potential to negatively impact product performance. Lastly, Dr Abend stated that BCS-based biowaivers are supported by comparative dissolution testing for class 1 and 3 drugs under certain conditions (per ICH).

The most common approach to assess dissolution profile similarity is the similarity factor, f_2. Introduced by Moore and Flanner in 1996, this mathematical approach is used to decide if two profiles are sufficiently similar in support of manufacturing changes or biowaivers. Similarity factor analysis has since been applied throughout the pharmaceutical industry and regulatory agencies. Unfortunately, health authorities are not aligned on the conditions under which similarity testing is conducted nor the acceptance criteria (albeit usually $f_2 \geq 50$ is typically considered acceptable). In case f_2 cannot be applied due to high variability in the amount of drug dissolved at individual sampling timepoints, agencies offer other mathematical approaches.

Interestingly, f_2 does not allow for type 1 error control (i.e., the risk of declaring profile similarity when profiles are dissimilar), and the use of superior statistical methods is not allowed. The fundamental problem with any profile similarity assessment is not the mathematical treatment of the data, but the discretionary power of the in vitro dissolution method to accurately assess how product

changes impact the in vivo performance. Without a clear link to in vivo performance, a dissolution test has unknown clinical relevance, and one cannot be certain that two profiles that are similar based on mathematical evaluations are equivalent in vivo. Likewise, two product variants with dissimilar in vitro profiles according to f_2 or other statistical tests may have similar in vivo performance. To overcome these fundamental challenges, a group of scientists (Abend, Hoffelder et al) developed a decision tree and best practices when dissolution data are used to assess the impact of manufacturing changes on product quality. The question at the core of the decision tree is whether or not an in vivo link between dissolution and pharmacokinetic point estimates exists. When this link has been established, then the dissolution method is clinically relevant, and decisions can be made based on comparing new dissolution profiles (after manufacturing changes) with the profiles used to establish the dissolution specification. If the dissolution profile representing a new manufacturing process falls within an acceptable dissolution safe space, then products made under these conditions are unlikely to negatively influence in vivo performance. However, if a safe space does not exist, then appropriate statistical methods with type 1 error control should be used.

Next, Dr. Michael Daniel Lucagbo gave a talk about statistical assessment of dissolution similarity. Dissolution profile comparisons are important in evaluating postapproval changes. Such comparisons should be based less on subjective assessments and more on scientific evidence and rigorous statistical procedures. Dr. Lucagbo presented some statistical approaches to assess dissolution similarity. Let μ_1=(μ_11,...,μ_1p)' and μ_2=(μ_21,...,μ_2p)' denote the population (p) mean values of the dissolution profiles of the test and reference products. When comparing these two dissolution profiles, regulatory guidance emphasizes f_2, given below.

$$f_2 = 50 \log_{10} \left(\frac{100}{\sqrt{1 + \frac{1}{p} \sum_{t=1}^{p} (\mu_{1t} - \mu_{2t})^2}} \right)$$

The similarity factor is a monotone function of the so-called Euclidean distance (ED), whose formula is shown below. Consequently, f_2 essentially provides the same information as the ED.

$$ED = \sqrt{\sum_{t=1}^{p} (\mu_{1t} - \mu_{2t})^2}$$

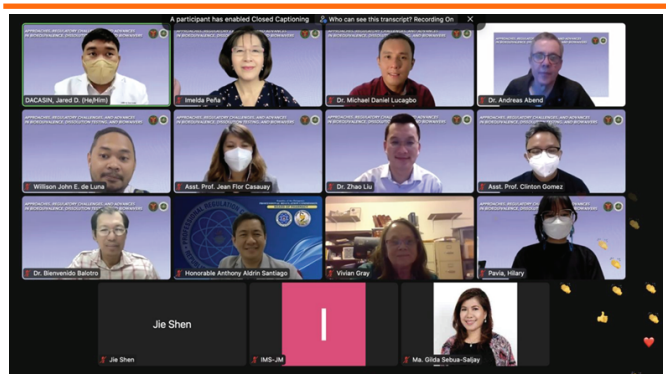
Dr. Lucagbo also cited other methods to compare dissolution profiles besides f_2 that are available. For example, another method that depends on the ED is the quadratic mean difference (QMD), which is computed as QMD $= \sqrt{(1/p)\,\mathrm{ED}^2}$. Hoffelder et al. provide in-depth discussion of model-independent statistical methods to evaluate similarity.

Instead of the ED as a measure of distance, many statisticians prefer the (squared) Mahalanobis distance (MD), which is computed as shown below:

$$MD = (\boldsymbol{\mu}_1 - \boldsymbol{\mu}_2)' \, \boldsymbol{\Sigma}^{-1} \, (\boldsymbol{\mu}_1 - \boldsymbol{\mu}_2)$$

where Σ is the common covariance matrix. Dr. Lucagbo provided references for the statistical tests for dissolution profile similarity using MD (e.g., Tsong et al.). For this test, rejection of the null hypothesis is an indication of similarity of dissolution profiles. Another reference is Wellek, who also provides an exact MD-based statistical test for similarity under a multivariate normal framework.

Dr. Liu moderated the panel discussion at the end of the second day.



Day 2 virtual workshop attendees and speakers.

Day 3: Advances

Dr. Imelda G. Pena gave a synopsis of the second day and opened the third day of the event.

Dr. Shen gave the first presentation, "In Vitro-In Vivo Correlation (IVIVC) of Complex Dosage Forms." She introduced the IVIVC and its categories, history, and current landscape. She discussed IVIVC development and validation in detail, highlighting considerations for formulation selection, in vitro dissolution method development and modelling, and in vivo study design and deconvolution techniques. Dr. Shen shared two case studies: 1) an extended-release formulation (upadacitinib, a BCS class I compound for rheumatoid arthritis) and 2) a long-acting suspension product (INVEGA SUSTENNA, a schizophrenia treatment), exemplifying the key steps of IVIVC development and validation. Dr. Shen ended her talk with recent exciting advances in demonstrating a level A

IVIVC for complex long-acting polymeric parenterals with an example of risperidone poly(lactic-co-glycolic acid) (PLGA) microspheres in a rabbit model.

Dr. Sandra Suarez-Sharp (Simulations Plus) gave the next presentation on "Mechanistic modeling as an In Vivo Linkage to In Vitro Dissolution Methods." Incorporating QbD principles into the pharmaceutical industry has broadened the scope of dissolution testing beyond its traditional role of supporting biowaivers after significant CMC changes, as outlined in the SUPAC guidance. The significance of whether an attribute, parameter, or in-process control is deemed critical to a drug product performance is contingent upon meeting dissolution criteria, irrespective of wide variations in those specific attributes/parameters. Consequently, dissolution testing assumes a pivotal role that cannot and should not be replaced solely by controlling critical material attributes (CMAs) and CPPs. This necessity arises because dissolution testing stands as the sole in vitro assessment capable of probing the extent and rate of in vivo drug release.

Despite the well-established value of dissolution testing in drug product development, its recognition as a key facilitator of "enhanced" drug product understanding often encounters obstacles due to uncertainties surrounding predictive ability and clinical relevance. This challenge is particularly pronounced for drug products containing BCS class 2 and 4 compounds and modified-release formulations. Dr. Suarez's presentation addresses the transition from discriminative to bio-predictive dissolution methods, acknowledging the need to establish relationships between critical attributes/process parameters, dissolution, and systemic exposure. Failure to comprehend these relationships can lead to overly broad, excessively stringent, or entirely irrelevant drug dissolution acceptance criteria, hindering our ability to determine whether the method is overdiscriminating, thereby imposing hurdles on companies, or under discriminating, thereby posing risks to patients.

A recommended approach involves initiating or considering the implementation of risk assessment and prior knowledge to identify potential CMAs, CPPs, and critical formulation variables (CFVs) that are likely to impact both in vitro and in vivo drug product performance. This approach ideally includes design of experiments (DoE) studies to confirm the level of risk and, more crucially, employing dissolution as an endpoint in these studies to identify formulation variants with extreme dissolution profiles. These variants can then be evaluated in relative BA/BE studies to establish the essential in vivo link and determine the level of rank order (over/under discriminating method), crucial for constructing an in vitro-in vivo relationship (IVIVR) or IVIVC and ultimately defining a safe space.

Although IVIVCs have been considered the gold standard for establishing the essential link to bolstered dissolution testing, the adoption of physiologically based biopharmaceutics modeling (PBBM) has gained traction within the scientific and regulatory communities for such roles. The strength of PBBM lies in its ability to leverage extensive data generated across the product development process, including biopharmaceutics, in vitro, and clinical pharmacokinetic data, to create a physiologically meaningful connection between in vitro and in vivo aspects. Coupled with virtual BE, this approach results in the establishment of a safe space. Consequently, this approach facilitates the construction of the crucial in vitro-in vivo link and empowers dissolution testing to set its boundaries, permitting the rejection of batches falling beyond this safe space. Ultimately, this leads to clinically relevant and bio-predictive dissolution testing, and thus manufacturing flexibility.

PBBM serves as a catalyst in solidifying the essential in vitro-in vivo link by seamlessly integrating formulation and manufacturing factors with dissolution to forecast their impact on systemic exposure. PBBM fosters a mechanistic understanding of in vivo drug release and its interaction with physiology, culminating in the development of IVIVRs. This approach offers a simplified route to biowaivers, particularly for IR drug products, where the success rate of IVIVCs has historically been limited. Dr. Suarez-Sharp's presentation underscores the importance of a fundamental shift in the pharmaceutical industry, promoting an approach to drug development that prioritizes early bio-predictive measures, with PBBM taking on a pivotal role in this transformation.

The final presentation was given by Dr. Alicia P. Catabay (De La Salle Medical and Health Sciences Institute [DLSMHSI]), "BA/BE Studies for Drug Development and In Vivo Drug Product Performance Evaluation." Dr. Catabay talked about the Center for Biopharmaceutical Research (CBR) at DLSMHSI. The CBR was established primarily to support the Philippine government's National Drug Policy and, in particular, to provide quality assurance by proving BE testing of locally manufactured pharmaceutical products in comparison with innovator drugs or those drugs already available in the market. Instituted in 1997, the CBR was originally a tripartite project of the Department of Pharmacology of the DLSMHSI College of Medicine, Novartis Inc., and the Bureau of Food and Drugs (now known as the Philippine FDA).

Dr. Catabay emphasized the role played by the CBR in ensuring the quality and efficacy of generic medicines. Currently, the BA/BE unit is the only fully independent academic-based BE testing laboratory in the Philippines, operating under Good Clinical Practices (GCP) and Good Laboratory Procedures (GCP). It was the first of five centers to be accredited by the Philippine FDA for BE studies, and it has garnered the Center of Excellence award given by the United States Pharmacopeia. The BA/BE unit conducts six to eight studies per year and boasts a 24-bed testing facility, recently upgraded in July 2019. Due to the pandemic, operation of the CBR was put on hold until it reopened in 2022, when partner laboratories started sending in requests for BA/BE testing. Today, the CBR is a primary center for establishing the BE of locally made drugs, ensuring the safety, efficacy, and quality of these drugs that are more accessible to the Filipino public.

The bioanalytical component of BA/BE testing is outsourced because there is no accredited bioanalytical laboratory in the Philippines. Samples are sent to Indonesia, Singapore, and Malaysia. The CBR is exploring partnerships with different funding agencies to establish a bioanalytical laboratory.

Asst. Prof. Ethel Andrea C. Ladignon facilitated the open forum and gave a synopsis of the webinars.

Dr. Leonel Santos gave the closing remarks and thanked all the presenters and participants who joined the 3-day virtual workshop.

Day 3 virtual workshop attendees and speakers.

ACKNOWLEDGEMENTS

The authors thank the AAPS-IVRDT community and the University of the Philippines Manila College of Pharmacy for co-hosting the online webinar series. We are grateful to the University Office of International Linkages for the hosting grant. Special thanks to the UPM-Information Management Service for technical assistance and to our three student assistants, Jared Dacasin, Hilary Grace Pavia, and Bea Louisse Truya.

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dx.doi.org/10.14227/DT300423P260

Highlights from 2022 AAPS 360–In Vitro Release and Dissolution Testing Community Annual Meeting

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The AAPS In-vitro Release and Dissolution Testing (IVRDT) Community met for their annual in-person meeting at 2022 PharmSci 360 conference in Boston. The meeting was attended by more than 50 active members of the community who participated in an engaged and lively meeting. At the beginning, the community chair Dr. Andre Hermans summarized the work and accomplishments of the IVRDT community in 2022.

Preceding the PharmSci 360 meeting, the IVRDT community held a successful virtual workshop in collaboration with the AAPS Stability community on "Dissolution Best Practices and International Harmonization." The workshop focused on dissolution testing requirements between different pharmacopoeias such as the Chinese Pharmacopeia (ChP), United States Pharmacopeia (USP), European Pharmacopeia (EP), and Japanese Pharmacopeia (JP). In the second part of this workshop, subject matter experts shared dissolution best practices with respect to method development and hydrodynamic considerations in apex vessels and USP dissolution apparatus 1 (basket).

To continue the collaboration between the Society for Pharmaceutical Dissolution Science (SPDS) and the AAPS IVRDT community, a 2.5-day in-person conference on "Dissolution Science: Principles and Applications" was held in September 2022, with over 130 attendees. A vast array of dissolution-related topics were discussed to highlight complexities and recent trends in dissolution science. Topics included biorelevant dissolution, physiologically based biopharmaceutics modelling (PBBM), regulatory aspects, fully automated testing methods, and long-acting injectable formulations.

The community announced two outreach workshops at the meeting, which were successfully held since then in November 2022 with the Jagiellonian University in Poland and in February 2023 in collaboration with the University of Philippines Manila. These workshops continue the long-standing efforts of the community and enable deep scientific discussions around the globe to increase awareness and knowledge of dissolution science.

Following the presentation at 2022 PharmSci 360, the IVRDT community brainstormed themes and activities for 2023. Of high interest were the topics of dissolution and data integrity, in-vivo predictive dissolution systems, challenges for amorphous solid dispersions, novel routes of drug delivery, pediatric dosage forms (administered with food), virtual bioequivalence measurements, and upcoming updates to *USP* General Chapter <711> Dissolution.

Meeting attendees included: Andre Hermans, Vivian Gray, Nicholas DeWeerd, Penny Peterson, Maria Cruanes, Agnes Zhao, Sherwin Xie, Karl Box, Scott Stephenson, Jeff Kiplinger, Keith Hamman, Martin Brandl, Raafat Fahmy, Ahmed Ibrahim, Yuly Chiang, Juan Song, Yogesh Chandhari, Kailas Thakker, Roshni Patel, Sanjani Ray, Akira Hattori, Ming Li, Karl Wagner, Mark Liddell, Amit Bansal, Michael Zaleski, Dan Spisak, Ken Boda, Lee Dowden, Rishabh Bahl, Susann Bellmann, Jonas Eriksen, Marina Navas Bachiller, Ana Coutinho, Alexandra Taseva, Marilyn Martinez, Anthony DeStefano, George Wang, Dave Kwajewski, Chris Rego, Vivek Shaw, Deidre D'Arcy, Zhao Lui, Alger Salt, Ishai Nir, Tahseen Mirza, Rachel Guo, Himanshu Gandhi, Michel Magnier, and Patrick Ballmer.

*Corresponding author

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Q+A Question & Answer Section

*The following questions have been submitted by readers of Dissolution Technologies. Margareth R. Marques, Ph.D., and Mark Liddell, Ph.D., United States Pharmacopeia (USP), authored responses to each of the questions. *Note: These are opinions and interpretations of the authors and are not necessarily the official viewpoints of the USP. E-mail for correspondence: mrm@usp.org.*

Q ***USP*** **general chapter <711> Dissolution states "The water bath or heating device permits holding the temperature inside the vessel at 37 ± 0.5 °C during the test and keeps the bath fluid in constant, smooth motion." Is there any control that should be performed during the test to verify the temperature? We are doing a pre-test in the vessel to control the temperature and a quality control test every 3 months to ensure that the water bath is calibrated to 37 ± 0.5 °C.**

A The recommendation is to check the temperature before starting the test in each vessel. Once the medium in each vessel is at the right temperature, the sensor/thermometer should be removed from the vessel. The thermometer/sensor cannot remain in the vessel during the test. Some labs check the temperature of each vessel after finishing the test. Also, the temperature is part of the periodic equipment verification.

Q **In the Dissolution Guideline Document for the new Dissolution Performance Verification Standard (DPVS) (available at https://www.usp.org/resources/compendial-tools) there is a recommendation to allowing staggered start, if possible. Should staggered start also be applied to routine sample testing?**

A Yes, if the sampling is done manually, the recommendation is to stagger the introduction of sample to allow sufficient time to collect the sample and filter it before moving to the next vessel so that sampling is performed within the time tolerance of ± 2%, as stated in *USP* general chapter <711> Dissolution.

Q **How should the disintegration results be expressed, the average of 6 units or the highest disintegration time?**

A The results are expressed for each individual unit tested.

Q **In the *USP* monograph for Isosorbide Mononitrate Extended-Release Tablets, Dissolution Test 1, it states that "tablets are placed in a metal helix prepared by winding 10 in. of a 0.8-mm stainless steel wire around a 9/32-in shaft and pulling the coils to form a helix 1-in. long." Does the USP store sell this metal helix, or should it be prepared by winding a stainless-steel wire?**

A USP does not sell any accessories used in dissolution testing. The instructions on how to make the metal helix sinker can be found in *USP* general chapter <1092> The Dissolution Procedure – Development and Validation.

Q ***USP*** **general chapter <701> Disintegration states "*The use of disks is permitted only where specified or allowed in the monograph.*" Can you elaborate or provide additional criteria regarding use of disks and the applicable formulation types?**

A The use of disks in the disintegration testing is defined experimentally using the samples under evaluation. Their use is defined in a case-by-case approach.

Q **We are qualifying our dissolution apparatus that has eight positions. After reading *USP* general chapters <1058> Analytical Instrument Qualification and <711> Dissolution, we considered that dissolution equipment is in group C in <1058>, requiring installation qualification, operation qualification, and performance verification. We think that the design qualification is not necessary for dissolution equipment because they are well described in <711>. Do you have any comments on this reasoning?**

A The *USP* general chapter <1058> provides recommendations on instrument qualification. It is up to your lab to decide how to classify the equipment. Even though design qualification may not be applicable, you need to know how the samples are inserted in the equipment and if it is possible to stagger both the sample introduction and movement of the shafts, which is important for manual sampling. Also, you need to consider if the sampling is going to be manual, semi-automated, or automated. Other aspects may be considered depending on the use of the equipment. In addition, you need to select the place where you are going to install the equipment. It must be level and free of vibration.

Q In *USP* general chapter <711> Dissolution and in the certificate provided with the USP Dissolution Performance Verification Standard – Prednisone RS, we found the description of the performance verification test used for the dissolution apparatus. Is the test used for performance qualification of the dissolution apparatus? Should the same procedure be used for periodical verification of the equipment? Could a finished product be used instead?

A The qualification procedure described in <711> Dissolution and in the USP RS certificate should be used for qualification of dissolution apparatus 1 and 2, both the initial test at equipment installation and for all subsequent tests.

Q For dissolution equipment with eight positions, which acceptance criteria should be used for performance verification, the criteria for six or eight positions?

A It is up to your lab to decide as it depends on how the equipment is going to be used. Consider if you will use all eight positions for samples or will six positions for samples and two positions for pre-warmed dissolution medium or placebo.

Q+A

Every issue of *Dissolution Technologies* features a Question and Answer section. This section is designed to address general dissolution questions submitted by our readers.

Please send your questions to:
Attn: Q&A
9 Yorkridge Trail, Hockessin, DE 19707
Email: vagray@rcn.com
Submit via our website:
www.dissolutiontech.com

Calendar of Events

November 13–14, 2023

Introductory GastroPlus® - Basic Concepts of PBBM
Location: University of São Paulo, São Paulo, Brazil
Registration: https://www.simulations-plus.com/events/introduction-to-gastroplus-basic-concepts-of-pbbm/

November 13–15, 2023

Eastern Analytical Symposium and Exhibition
Location: Crowne Plaza Princeton-Conference Center, Plainsboro, NJ, USA
For information, visit eas.org

November 13–15, 2023

Introductory GastroPlus® Workshop
Location: Tokyo, Japan
Registration: https://www.simulations-plus.com/events/introductory-gastroplus-workshop-tokyo-japan/

November 16–17, 2023

Advanced GastroPlus®DMPK and Clinical Pharmacology Workshop
Location: Tokyo, Japan
Registration: https://www.simulations-plus.com/events/advanced-gastroplus-workshop-tokyo-japan/

November 16–17, 2023

Advanced GastroPlus® Pharmaceutical Development Workshop
Location: Tokyo, Japan
Registration: https://www.simulations-plus.com/events/advanced-gastroplus-pharmaceutical-development-workshop-tokyo-japan/

November 23, 2023

Dissolution Discussion Group Quarterly Online Meeting—Dissolution Qualification: The PQ vs MQ debate. What's right for your lab?
Location: DDG Online Meeting at 10:30 am ET
Registration: https://www.agilent.com/chem/dissolution-webinars

November 28–December 1, 2023

GastroPlus® Advanced Pharmaceutical Development
Location: Online
Registration: https://www.simulations-plus.com/events/gastroplus-advanced-workshop-pharmaceutical-development/

December 4, 2023

Complimentary Introduction to GastroPlus® Workshop
Location: Online
Registration: https://www.simulations-plus.com/events/complimentary-introduction-to-gastroplus-workshop-12/

On Demand Events

- ***Simplifying Dissolution Automation with In-Situ FIber Optic UV On Demand***
  https://www.distekinc.com/watch/webinar-simplifying-dissolution-automation-with-in-situ-fiber-optic-uv/
- ***Clarifying 21 CFR Part 11 & Data Integrity Requirements for Dissolution Testing On Demand***
  www.distekinc.com/watch/clarifying-21-cfr-part-11-and-data-integrity-for-dissolution-testing/

- ***Ocular Administration (OCAT™) in GastroPlus® On Demand***
  https://www.simulations-plus.com/events/gastroplus-additional-dosage-routes-workshop-ocular-administration-ocat-virtual/

- ***Oral Cavity Administration (OCCAT™) in GastroPlus® On Demand***
  https://www.simulations-plus.com/events/gastroplus-additional-dosage-routes-workshop-oral-cavity-administration-occat-virtual/

- ***Pulmonary Administration (PCAT™) in GastroPlus® On Demand***
  https://www.simulations-plus.com/events/gastroplus-additional-dosage-routes-workshop-pulmonary-administration-pcat-virtual/

- ***GastroPlus® ADR – 4 Course Bundle (TCAT™ / OCAT™ / OCCAT™ / PCAT™)***
  https://www.simulations-plus.com/events/gastroplus-adr-4-course-bundle-tcat-ocat-occat-pcat/

- ***GastroPlus® ADR – 5 Course Bundle (TCAT™ / OCAT™ / OCCAT™ / PCAT™ / Injectables)***
  https://www.simulations-plus.com/events/gastroplus-adr-5-course-bundle-tcat-ocat-occat-pcat-injectables/

- ***Transdermal Administration (TCAT™) in GastroPlus®***
  https://www.simulations-plus.com/events/gastroplus-additional-dosage-routes-workshop-transdermal-administration-tcat-virtual/

- ***Injectables (IM, SQ, IA) in GastroPlus® Including Biologics and LAIs***
  https://www.simulations-plus.com/events/gastroplus-additional-dosage-routes-workshop-injectables-incl-lai-biologics-virtual/

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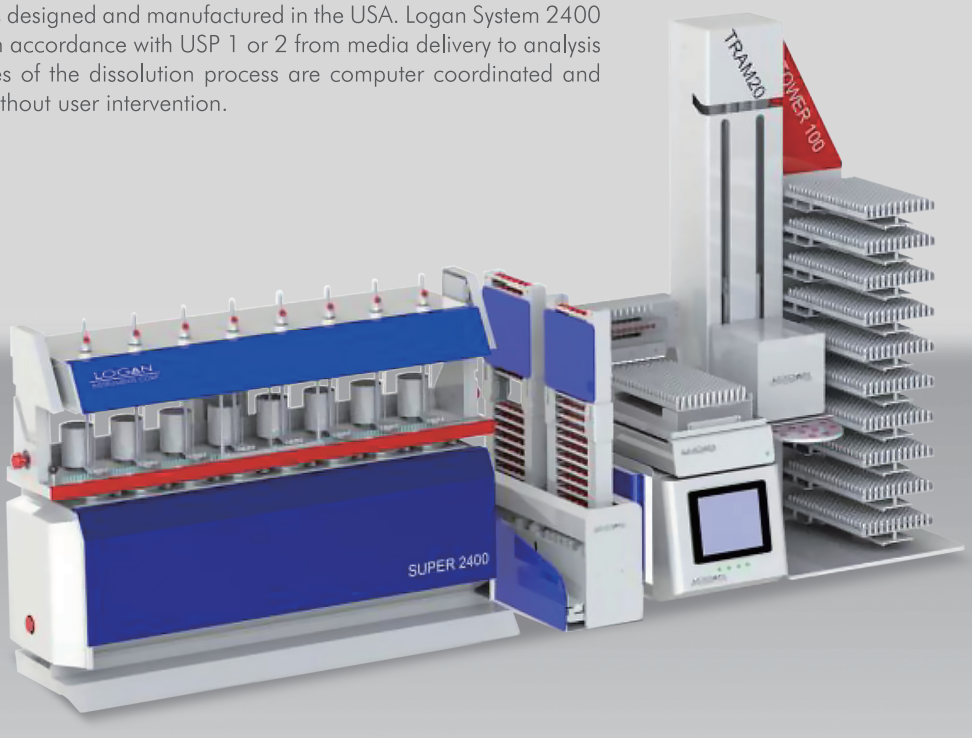
Logan Fully Automated Dissolution System

The Logan PELogan System 2400 is the only fully automated dissolution testing system that is designed and manufactured in the USA. Logan System 2400 can run 10 batches in accordance with USP 1 or 2 from media delivery to analysis unattended. All stages of the dissolution process are computer coordinated and carried out entirely without user intervention.



This system precisely delivers, preheats, and degasses media into 8 dry-heat vessels removing the added complication of a water bath. Each vessel has bottom and side cameras to record the dissolution for subsequent viewing and result verification. After the test is over, the vessels automatically empty the media, they are then sprayed, washed, and blow-dried, ready for the next 9 batches of test samples.

Logan System 2400 is equipped with two types of filter changers. The Super 2400 dissolution tester includes an automated filter tip changer for online UV analysis. An additional membrane filter changer is available for sample collection for offline HPLC analysis.

Online UV analysis

Filter changer for collection of samples for offline HPLC analysis

PVT 800

Logan PVT 800 toolset performs and records dissolution Performance Validation Testing (PVT) electronically, removing the uncertainties of manual recording. Logan PVT 800 toolset includes a temperature sensor, speed detector, vertical/horizontal, height, vibration, and centering gauges, etc. The PVT report is displayed on the PC screen, the data can be recorded in the hard drive and printed. With this advanced toolset, user intervention in the data-transferring process is eliminated. Logan PVT 800 toolset further improves compliance with FDA CFR 21 part 11.

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Industry News

Copley Launches a New Blog for the Pharmaceutical Testing Community

Building a 'go to' resource for reliable, educational material.

Nottingham, UK: Copley Scientific has launched a blog with the aim of building a trusted, educational resource for anyone involved with pharmaceutical testing. Via regular posts from company experts, the blog will cover testing for all types of pharmaceutical products: tablets and capsules, transdermals, semisolids, suppositories and, of course, orally inhaled and nasal drug products (OINDP), a field in which Copley leads the world. The move reflects the company's commitment to exemplary customer support and builds on an established track record of high-quality publications such as the renowned Inhaler Testing brochure. Register now (copleyscientific.com/join-our-mailing-list) to receive regular updates from the start.

"We're excited about the blog," says Jamie Clayton, Managing Director, "and we hope that it will prove valuable. Our goal is to provide informative content in key areas in a digestible format. Each blog will take just a few minutes to read but will hopefully provide something novel and interesting, whatever your current level of expertise. We're especially keen to set down some of the basics to help those that are new to pharmaceutical testing since it can be difficult to find clear, informative, and reliable material. We hope to make it easier."

The first blog is already live and new posts will be added on a regular basis. There will be planned themes such as dissolution testing and cascade impaction but also ad hoc posts from conferences, for example, or triggered by changes in regulatory practice. Sharing the knowledge that resides within the company to help readers enhance testing practices is the overarching aim.

"The blog provides us with a flexible communication channel," says Clayton "that we can use to speak to customers in the same way we would at say a one-to-one training session. As we progress you can expect Q&As and top tips alongside core, evergreen pieces on the fundamentals of testing. We'll also be putting the spotlight on successes in the community, notably advances in the flourishing OINDP arena, and responding to issues that are concerning or challenging people. We hope you'll find the blog a good read and join us regularly."

Introducing the voices of the Copley blog: Jamie Clayton (Managing Director), Matthew Fenn (Head of Business Development), Clair Brooks (Applications Specialist), Ben Bradley (Head of Product Development)and, , Imran Haneef (Business Development Manager).

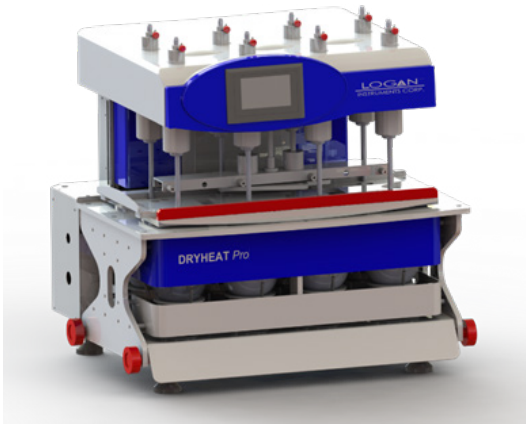
Logan Instruments Announces Dry Heat Pro Series Dissolution Tester

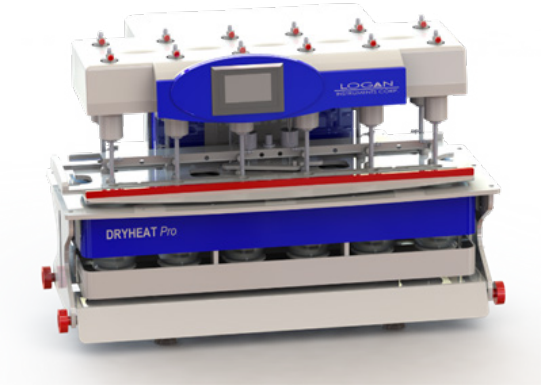
Logan Instruments Corp. is proud to announce the next evolution in dissolution apparatus 1, 2, 5, and 6.

The Dry Heat Pro Series Dissolution Tester replaces the traditional water bath. Each vessel has three-zone, contemporary, dry heat elements. The unique design ensures there is no cold zone at the bottom of the vessel. The three dry heat elements can be selected for optimal heating of any volume from 100 to 1000 mL. Dry heating ensures more rapid heating of the vessel media than can be achieved with a water bath. Vessel wall sensors protect against thermal shock, so standard vessels can be used. The sensors also maintain accurate and consistent temperature throughout each vessel for the duration of the test.

The Dry Heat Pro Series Dissolution Tester is available in 8, 12, 15, or 18 vessel configurations. All models comply with the requirements for R&D and QC. The larger capacity systems allow multiple QC batches to be run simultaneously.

Logan offers these models with up to three optional infra-red cameras for each vessel. Infrared imaging allows the study to run in complete darkness, avoiding the adverse effect of light on the test compound. One camera beneath the vessel, one on the side, and a third inside the shaft. These images are recorded for subsequent review and can help explain anomalous results.





Another new introduction to these models is in-line UV analysis with fiber optic probes. UV can also be measured online by sampling through Logan's parallel, 8 flow-cell spectrophotometers. As with all Logan dissolution systems the samples can also be automatically collected for off-line analysis.

To further advance your research the new dissolution apparatus connects directly to Logan's PERMETRO to economically streamline bioequivalence studies.

For more information about the new generation Dry Heat Pro Series Dissolution Tester please visit http://www.loganinstruments.com or contact us at infoDT@loganinstruments.com

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AAPS Announces 2023 Awards Recipients

Awards Program Recognizes Research and Leadership in Pharmaceutical Science

Arlington, VA—AAPS is proud to congratulate the recipients of the 2023 Awards Program. AAPS Awards celebrate scientists who are role models in the pharmaceutical science community. Each year, scientists volunteering through the AAPS Awards Committee review nomination submissions and select individuals whose scholarship, leadership, and service exemplify the highest personal and professional achievement.

"We are delighted to unveil the distinguished recipients of this year's AAPS Awards, whose exceptional contributions have brought about a transformative impact in the pharmaceutical community. Their unwavering dedication to advancing pharmaceutical academia, research, industry, and regulatory fields has set unprecedented standards of excellence in healthcare," AAPS 2023 Awards Committee Chair and Regents Professor and Director at Texas A&M University School of Medicine Doodipala Samba Reddy, PhD, R.Ph., FAAPS, said. "These exceptional individuals embody the spirit of ingenuity, marked by groundbreaking discoveries and revolutionary innovations that have significantly influenced patient care and the development of new drugs and products.

"We extend our heartfelt congratulations to this year's awards recipients and wish them continued success in their endeavors to further advance our thriving pharmaceutical community."

The 2023 AAPS Awards recipients are listed below.

Distinguished Scientist Award
Mansoor Khan, R.Ph., PhD, FAAPS, Regents Professor, Vice Dean, and Director of the Formulations Design and Development Core Laboratory at the Irma Lerma Rangel School of Pharmacy, Texas A&M University, is recognized for his wide-ranging and international impact on the pharmaceutical sciences. His work is broad, spanning education, R&D, and other areas. He also works closely with regulatory authorities. "You find his fingerprints everywhere," one Awards Committee member said.

Global Leader Award
Allen Templeton, PhD, FAAPS, Vice President, Pharmaceutical Sciences and Clinical Supply, Merck Research Laboratories, is recognized for the international impact of his science, and the bridge he has formed by his work in industry and instruction in academia. According to one of his nominations, Dr. Templeton's "impact on human health in developing numerous lifesaving medicines, his significant service and leadership roles in the profession, and his demonstrable contributions toward global outreach make him fully qualified of receiving the AAPS Global Leader Award. Dr. Templeton is a global leader by every definition."

Distinguished Service Award
Vivian Gray is recognized for her long history of service to AAPS, which includes assisting in the development of several programs such as the AAPS course, "Dissolution of Solid Oral Dosage Forms" and leading the formation of the AAPS In Vitro Release and Dissolution Testing (IVRDT) Community.

Emerging Leader Award
Raman Bahal, PhD, Associate Professor, Department of Pharmaceutical Sciences, University of Connecticut, is described as a "rising star" in the pharmaceutical sciences community, receiving tenure at the University of Connecticut after only 4 years.

"Dr. Bahal is an exceptionally gifted organic chemist with particular skill in synthesizing peptides and nucleic acid analogs," wrote one supporter in a nomination. "He is a leader in nucleic acid-based chemistry and its delivery strategies. He has a reputation, both nationally and internationally, as an outstanding pharmaceutical chemist."

Outstanding Manuscript Awards
"Cancer Immunotherapy Update: FDA-Approved Checkpoint Inhibitors and Companion Diagnostics," *AAPS Journal*, Julianne D. Twomey, PhD, and Baolin Zhang, PhD

"Polyvinyl Alcohol/Chitosan Single-Layered and Polyvinyl Alcohol/Chitosan/Eudragit RL100 Multi-layered Electrospun Nanofibers as an Ocular Matrix for the Controlled Release of Ofloxacin: an In Vitro and In Vivo Evaluation," *AAPS PharmSciTech*, Shahla Mirzaeei, PhD, Shiva Taghe, PhD, Kofi Asare-Addo, PhD, and Ali Nokhodchi, PhD

"Molecular, Solid-State and Surface Structures of the Conformational Polymorphic Forms of Ritonavir in Relation to their Physicochemical Properties," *Pharmaceutical Research*, Chang Wang, PhD., Ian Rosbottom, PhD, Thomas D. Turner, PhD, Sydney Laing, Andrew G. P. Maloney, PhD, Ahmad Y. Sheikh, PhD, Robert Docherty, PhD, Qiuxiang Yin, PhD, and Kevin J. Roberts.

Alice E. Till Advancement of Women in Pharmaceutical Sciences Recognition
Former AAPS President Diane J. Burgess, PhD, FAAPS, Distinguished Professor of Pharmaceutics, University of Connecticut, is recognized for an extraordinary career as a scientist, both as a researcher who has authored many publications, and as a leader. She is a former president of both AAPS and the Controlled Release Society. Dr. Burgess is credited by her supporters with using her platform to develop both individual women and the organizations they work in, allowing her to train and mentor women as researchers, instructors, and leaders.

Student Chapter Awards
The University of Texas AAPS Student Chapter is recognized for making a significant impact on graduate student researchers at the school through numerous activities, including a welcome event, a Design of Experiments Workshop, a presentation by Dr. Kerry Empey of the University of Pittsburgh School of Pharmacy, and several events with researchers from around the world.

The University of Toronto AAPS Student Chapter is recognized for efforts to provide mentoring opportunities for students, as exemplified by the launch of the chapter's first mentorship program. The program connected approximately ten graduate students and post-doctoral fellows with guidance and advice from alumni mentors currently working in either academia or industry.

Best Abstract Award
This year, 72 of the hundreds of poster abstracts submitted to PharmSci 360 before the early submission deadline were recognized with the Best Abstract Award. These posters will be presented by their authors at the 2023 PharmSci 360, Oct. 22-25, in Orlando, FL. Currently, early poster abstracts can be viewed in the PharmSci 360 program.

Best Poster Award
The most scientifically impactful posters that will be presented at PharmSci 360 will be announced in August.

Award recipients were recognized at the 2023 PharmSci 360 in Orlando, FL, on Oct. 22, at the Orange County Convention Center. For more information about this event, visit www.aaps.org/pharmsci360.

Simulations Plus Releases ADMET Predictor® 11

New functionality, models, and partner data power predictive accuracy from the industry-leading machine learning platform

Lancaster, CA -- Simulations Plus, Inc. (Nasdaq: SLP), a leading provider of modeling and simulation solutions for the pharmaceutical and biotechnology industries, announced the release of ADMET Predictor® 11, its flagship machine learning modeling platform.

The latest version of ADMET Predictor includes:

- New industry partner data that more than doubles the number of ionization constants (pKa), leading to enhanced predictive accuracy and wider applicability of our S+pKa model
- New functionality to perform 3D virtual screening based on shape and pharmacophore-feature similarity
- New CYP inhibition (Ki) models to allow for rapid drug-drug interaction (DDI) risk assessment
- Significant enhancements to the AI-driven drug design (AIDD) module

“Amidst the growing adoption of machine learning in the pharmaceutical industry, there has been an influx of software platforms boasting machine learning capabilities,” commented Dr. Robert Fraczkiewicz, Research Fellow at Simulations Plus and project leader for the pKa collaborations. “However, it is important to recognize that rapid and reliable predictions cannot be achieved through machine learning alone. It necessitates training on premium, extensively curated datasets and the implementation of refined, time-tested algorithms. ADMET Predictor 11 stands out as the sole platform in the market that fulfills all these key criteria, setting a new benchmark for excellence in the field.”

“We continue to support our clients through the integration of machine learning with mechanistic modeling,” added Dr. Eric Jamois, Senior Director of Key Accounts and Strategic Alliances. “The latest advancements enable our users to complement high-throughput pharmacokinetic (HTPK) simulations with rapid assessment of drug-drug interaction (DDI) liabilities, powering the novel selection of clinical candidates. We are proud to deliver this cutting-edge version to our expanding user community and help propel drug discovery research to unprecedented heights.”

Visit the Simulations Plus website to learn more about ADMET Predictor 11.

Simulations Plus Receives New FDA Grant Award

Collaboration with regulatory, industry, and academic partners will support and accelerate the development and validation of workflows to conduct virtual bioequivalence studies

Lancaster, CA -- Simulations Plus, Inc. (Nasdaq: SLP), a leading provider of modeling and simulation solutions for the pharmaceutical, biotechnology, chemicals, and consumer goods industries, announced it has been awarded a new funded grant from the U.S. Food and Drug Administration (FDA). The grant will be used to validate and define best practices for physiologically based biopharmaceutics/pharmacokinetics (PBBM/PBPK) modeling workflows to simulate virtual bioequivalence (VBE) studies in support of regulatory biowaivers.

The scientific team at Simulations Plus, with partners from the FDA, industry, and academia, will apply public and proprietary datasets to validate the ability of GastroPlus® and other software to predict inter- and intra-subject variability when performing virtual population simulations. Enhancements to the existing VBE simulation engine within GastroPlus will be implemented, and best practices will be defined for VBE evaluation using mechanistic PBBM/PBPK approaches. The goal of this work is to inform regulatory decisions and guide innovator and generic drug developers in the design of VBE studies for distinct types of drug products.

Dr. Frederico Martins, Principal Scientist and LATAM Scientific Lead, PBPK Solutions, and Principal Investigator for this grant, said: "At the recent PBBM/PBPK workshop sponsored by the University of Maryland Center for Excellence in Regulatory Science and Innovation (M-CERSI) and the FDA, it became clear that regulatory agencies worldwide are actively promoting the increased adoption of VBE approaches in numerous ways. The many case studies presented at the workshop, nearly all of which utilized GastroPlus simulations, firmly reinforced GastroPlus as the leading software platform for drug product development. With this new award, we are poised to shape industry best practices and define innovative workflows for leveraging in vitro systems and in silico models to further reduce regulatory burden and minimize the need for human studies."

FDA scientific and program staff will actively collaborate with Simulations Plus and others from industry and academia. Dr. Martins, with assistance from Simulations Plus colleagues Dr. Maxime Le Merdy, Dr. Géraldine Cellière, and Mr. James Mullin, will coordinate all activities of the award.

"With a rich and enduring history of fruitful partnerships with the FDA, we have consistently demonstrated our commitment to collaboration and excellence in research and regulatory affairs," added Dr. Le Merdy, Associate Director, Research & Collaborations of PBPK Solutions. "Our mission with this award is clear: to set the industry standard and provide invaluable guidance for all companies navigating the regulatory assessment of new and generic formulations. By diligently striving to make all forthcoming enhancements accessible to industry, nonprofit organizations, and academic researchers, we will enable the advancement of modeling and simulation applications. This commitment ensures that the benefits extend not only to those within the scientific community but also reach patients worldwide."

Funding for this collaboration is made possible by the Food and Drug Administration through grant award 1U01FD007906-01. Views expressed in this press release do not necessarily reflect the official policies of the Department of Health and Human Services; nor does any mention of trade names, commercial practices, or organization imply endorsement by the United States Government.

Simulations Plus Chief Science Officer Dr. Viera Lukacova Honored as Fellow by the American Association of Pharmaceutical Scientists (AAPS)

Dr. Lukacova's outstanding contributions to pharmaceutical research and innovation to be recognized with seven others at AAPS PharmSci 360

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Lancaster, CA -- Simulations Plus, Inc. (Nasdaq: SLP), a leading provider of modeling and simulation solutions for the pharmaceutical and biotechnology industries, is proud to announce that Dr. Viera Lukacova, Chief Science Officer of the SLP Division, has been elevated to the status of Fellow within the American Association of Pharmaceutical Scientists (AAPS) and will be formally inducted at the annual PharmSci 360 meeting on October 22, 2023.

"Viera stands out as an eminent figure whose profound contributions have significantly shaped the landscape of physiologically based pharmacokinetics (PBPK) modeling," said John DiBella, President of the SLP Division at Simulations Plus. "Her scientific insights have advanced the capabilities of software platforms like GastroPlus®, DDDPlus™, and MembranePlus™ that scientists use every day to develop safe and effective treatments for patients worldwide. A tireless advocate, her impact also extends beyond research into the realms of publication, presentation, and education. Through her visionary leadership, pioneering research, and relentless dedication, she has elevated PBPK modeling to unprecedented heights, and we cannot wait to celebrate the well-deserved status she has achieved within AAPS at October's meeting."

"The Class of 2023 AAPS Fellows [...] were selected based on well-defined criteria for outstanding scientific accomplishments, significant and sustained impact in the pharmaceutical sciences, and exceptional service to the AAPS community," said AAPS 2023 Fellows Committee Chair Mandip Singh Sachdeva, PhD, FAAPS.

Joining Dr. Lukacova as 2023 AAPS Fellows are Drs. Shaukat Ali, Ben Boyd, Maria Croyle, Otilia Koo, Xiuling Lu, Wellington Pham, and Patrick Ronaldson.

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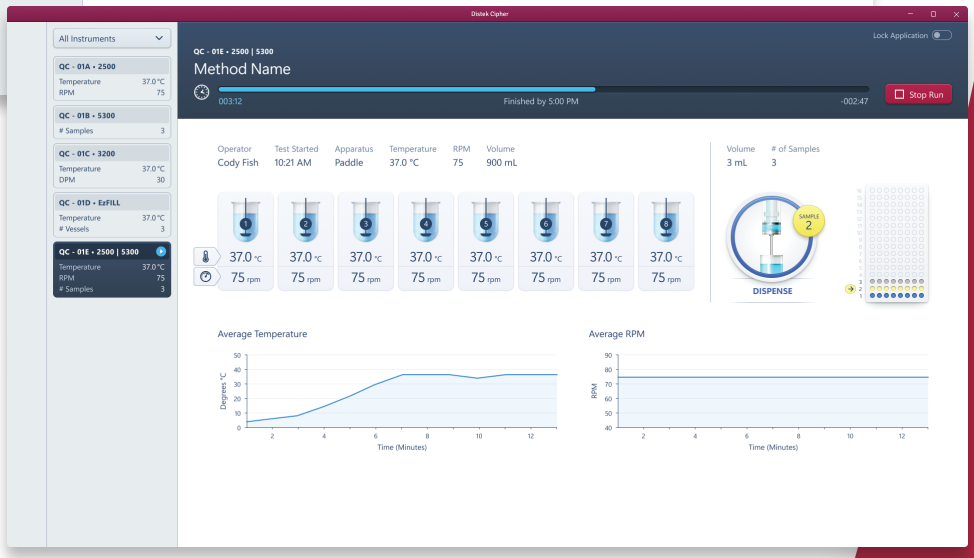

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